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# The Metabolism of Chicken Tumors[1]

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## INTRODUCTION

Since the studies of Warburg and associates (*2, 3*), carried out during the years 1923–30, much information has accumulated leading to the formulation of a fairly well defined concept of tumor metabolism (cf. Dickens (*4*) and Burk (*5, 6*)). Warburg found that in general tumors were growing tissues with a comparatively low oxygen consumption that glycolyzed (produced lactic acid from glucose) under aerobic as well as anaerobic conditions. Subsequent work by Dickens and collaborators (*7–13*) and others has shown that this metabolism is further characterized by a respiratory quotient that is in general definitely below unity (0.75–0.90, ±), differing thereby from the metabolism of most of the other growing tissues, which, though they may also glycolyze, seldom possess a respiratory quotient markedly below unity.

The metabolism of chicken neoplasms has been studied very little, the leukoses not at all, and of the many tumors described only chicken tumor 1 of Rous, chicken tumor 9 of Murphy and Landsteiner (*14*), and the Mill-Hill fowl fibrosarcoma 1 of Begg (*15*). These were reported upon briefly and intermittently from 1925 to 1933, tumor 1 by Warburg (*2, p. 197*), Murphy and Hawkins (*16*), Crabtree (*17*), Dickens and Simer (*7, 8*), and Dickens and Greville (*10*); tumor 9 by Murphy and Hawkins (*16*); and the Mill-Hill fowl fibrosarcoma 1 by Dickens and Greville (*10, 13*). The series of new chicken tumors and leukoses described and made available by Furth and coworkers (*18–25*) during the past few years, and the new developments and refinements of manometric and chemical techniques introduced during the past 2 years by Summerson (*26*) and Barker and Summerson (*27*) have made the present studies possible, desirable, and timely.

Collateral histologic studies have been carried out which have enabled certain provisional correlations to be made between the metabolism and the microscopic appearance of the tumor tissues. This has provided for some understanding of the variability between different kinds of chicken tumors and between individual samples of a given strain of tumor, and has given rise to suggestions for more detailed studies of a similar nature of applicability to tumor metabolism investigations generally.

In general, the results obtained with the chicken tumors support and amplify the concept of tumor metabolism described above. The studies on the leukoses will be reported in a forthcoming paper.

## METHODS

Each experiment was set up to determine for a given sample of tissue the following manometric values: aerobic acid production ($Q^{O_2}_A$), respiratory carbon dioxide production ($Q_{CO_2}$), oxygen consumption ($Q_{O_2}$), and respiratory quotient (R. Q.= $Q_{CO_2}/Q_{O_2}$), for a 2-hour experimental period; and anaerobic acid production ($Q^{N_2}_A$), ordinarily for 4 hours by hourly periods; and the chemical values, aerobic and anaerobic lactic acid production ($Q^{O_2}_{LA}$) and ($Q^{N_2}_{LA}$). All Q values reported are in terms of cubic millimeters/milligram initial dry weight/hour in which 1 $mm.^3 = 1/22400000$ mol. The aerobic manometric determinations were carried out with the recently developed Summerson constant-volume differential manometer (*26*), and the anaerobic determinations with the ordinary simple constant-volume Warburg manometer. The vessels used with the Summerson manometer were single sidearm Dixon-Keilin vessels of about 18-cc. capacity, with the KOH wells located in the stopcock operated underneath. Vessels with two sidearms

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were ordinarily used on the Warburg manometers, one sidearm containing 0.3 cc. of 2.5 N HCl and the other 0.3 cc. of brom thymol blue indicator. For each type of determination duplicate measurements were generally made, and in many instances triplicate. In a representative number of experiments, the lactic acid contents of the manometer vessels were analyzed quantitatively by the chemical method developed by Barker and Summerson (*27*) from the original method of Miller and Muntz (*28*). Chemically analyzed lactic acid values ($Q_{LA}$) for the experimental period were thus made available for comparison with the corresponding manometric total acid values ($Q_A$). All experiments were carried out in a water bath at 37°–39°, usually 38° C.

## EXPERIMENTAL PROCEDURE

A detailed example of the manometric and chemical procedure is indicated in the protocol, which will serve as a guide for experiments to be reported not only in the present paper but also in similar papers to follow dealing with various aspects of tumor metabolism.

*Typical protocol of standard metabolism experiment* [1]

AEROBIC DATA

| Manometric data: | T. B.[2] | A | | C | | D | | E | |
|---|---|---|---|---|---|---|---|---|---|
| Manometer No. | | 32 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Vessel No. | | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Volume of medium—cubic centimeters | | | | | | | | | | |
| Volume of 2.5 N HCl (in sidearm) cubic centimeters | | .30 | .30 | .30 | .30 | .30 | .30 | .30 | .30 | .30 |
| $O_2$ vessel constant, $k_{O_2}$ [3] | | 1.30 | 1.29 | 1.29 | 1.22 | 1.22 | 1.19 | 1.19 | 1.14 | 1.14 |
| $CO_2$ vessel constant, $k_{CO_2}$ [3] | | 1.48 | 1.47 | 1.47 | 1.40 | 1.40 | 1.37 | 1.37 | 1.32 | 1.32 |
| Wet weight milligrams | | 0 | 152 | 151 | 153 | 154 | 153 | 152 | 153 | 153 |
| Dry weight (14.0 pct.) milligrams | | 0 | 21.3 | 21.1 | 21.4 | 21.6 | 21.4 | 21.3 | 21.4 | 21.4 |
| Scale readings (with T. B. till t=120): | | | | | | | | | | |
| t=−15 minutes | | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 |
| t=0 (acid tipped, vessels 1) | | 158 | 191 | 188 | 188 | 189 | 189 | 184 | 187 | 191 |
| t=5 minutes | | 159 | | 198 | | 201 | | 194 | | 202 |
| t=10 minutes | | 160 | | 208 | | 211 | | 202 | | 211 |
| t=15 minutes | | 162 | | 217 | | 221 | | 211 | | 222 |
| t=30 minutes | | 165 | (213) | 244 | (217) | 252 | (216) | 239 | (215) | 252 |
| t=45 minutes | | 166 | | 268 | | 278 | | 263 | | 276 |
| t=60 minutes | | 166 | | 289 | | 304 | | 284 | | 301 |
| t=120 (acid tipped, vessels 2) | | 168 | (213) | 369 | (217) | 386 | (216) | 364 | (215) | 384 |
| t=150 (readings differential) | | | 308 | 273 | 325 | 287 | 318 | 281 | 325 | 292 |
| t=180 (readings differential, KOH then added, vessels 1, 2) | | | 307 | 275 | 325 | 287 | 318 | 281 | 325 | 292 |
| t=240 (readings differential) | | | 336 | 175 | 341 | 178 | 339 | 179 | 369 | 188 |
| t=300 (readings differential) | | | 334 | 175 | 337 | 172 | 337 | 178 | 351 | 182 |
| $h$ reading, millimeters, $h_{O_2}+h_{CO_2}+h_A$ | | | | 171 | | 187 | | 170 | | 183 |
| $h_1$ reading, millimeters, $h_{O_2}+h_{CO_2}$ | | | | −29 | | −39 | | −32 | | −37 |
| $h_2$ reading, millimeters, $h_{O_2}$ | | | | −156 | | −166 | | −155 | | −173 |
| $X^{O_2}{}_A=(h-h_1)k_{CO_2}$, cubic millimeters produced | | | | 294 | | 316 | | 277 | | 290 |
| $X_{CO_2}=(h_1-h_2)k_{CO_2}$, cubic millimeters produced | | | | 187 | | 178 | | 168 | | 179 |
| $X_{O_2}=-(h_2)k_{O_2}$, cubic millimeters *consumed* [4] | | | | 201 | | 202 | | 184 | | 197 |
| $Q^{O_2}{}_A=X^{O_2}{}_A$/milligrams dry weight/hour cubic millimeters | | | | 7.0 | | 7.3 | | 6.5 | | 6.8 |
| $Q_{CO_2}=X_{CO_2}$/milligrams dry weight/hour cubic millimeters | | | | 4.4 | | 4.1 | | 3.8 | | 4.2 |
| $Q_{O_2}=X_{O_2}$/milligrams dry weight/hour cubic millimeters | | | | 4.8 | | 4.7 | | 4.3 | | 4.6 |
| R. Q.=$Q_{CO_2}/Q_{O_2}$ | | | | .92 | | .87 | | .88 | | .91 |
| Chemical data [5] (lactic acid) | $S_1$ | $S_2$ | | | | | | | | |
| Colorimetric readings $R_1$ | 97 | 216 | 128 | 256 | 236 | 262 | 114 | 226 | 115 | 232 |
| $R_2$ | 99 | 221 | 123 | 244 | 225 | 262 | 118 | 220 | 123 | 226 |
| Average colorimetric reading $R=(R_1+R_2)/2$ | 98 | 218 | 125 | 250 | 230 | 262 | 116 | 223 | 119 | 229 |
| R−B (F=40, B=18) | | | 107 | 232 | 212 | 244 | 98 | 205 | 101 | 111 |
| T, total LA, cubic millimeters/vessel | | | 220 | 478 | lost | 503 | 203 | 422 | 229 | 449 |
| N, net LA, cubic millimeters/vessel | | | | 258 | | | | 219 | | 220 |
| $Q^{O_2}{}_{LA}=N$/milligrams dry weight/hour, cubic millimeters | | | | 6.1 | | | | 5.2 | | 5.2 |

See footnotes at end of protocol.

ANAEROBIC DATA

| | | | | |
|---|---|---|---|---|
| Manometric data: | | | | |
| Manometer No. | | 5 | 3 | 4 | 6 |
| Vessel No. | | 35 | 33 | 34 | 36 |
| Volume of medium | cubic centimeters | 2.0 | 2.0 | 2.0 | 2.0 |
| Volume of 2.5 N HCl (sidearm) | cubic centimeters | .3 | .3 | .3 | .3 |
| Volume of brom thymol blue (second sidearm) | cubic centimeters | .3 | .3 | .3 | .3 |
| $CO_2$ vessel constant, $k_{CO_2}$ | | 1.35 | 1.39 | 1.34 | 1.33 |
| Wet weight | milligrams | 43 | 43 | 43 | 44 |
| Dry weight (14.0 pct.) | milligrams | 6.0 | 6.0 | 6.0 | 6.2 |
| $h$, net pressure change (corrected for T. B.), per given time interval, one to next—millimeters—$t$ = 0 (brom thymol blue tipped, pH read, acid tipped): | | | | | |
| $t$ = 15 minutes | | pH, 7.2 | 20 | 19 | 22 |
| $t$ = 30 minutes | | | 19 | 19 | 19 |
| $t$ = 45 minutes | | | 17 | 16 | 16 |
| $t$ = 60 (0–60 minutes in parentheses) | | | 19 (75) | 16 (70) | 16 (73) |
| $t$ = 120 minutes | | | 65 | 62 | 62 |
| $t$ = 180 minutes | | | 55 | 52 | 55 |
| $t$ = 240 (0–240 minutes, in parentheses; brom thymol blue tipped, pH read, acid tipped) | | | 48 (244) | 48 (232) | 44 (234) |
| | | pH | 6.8 | 6.8 | 6.8 |
| $X^{N_{2_A}} = hk_{CO_2}$ cubic millimeters acid produced per hour: | | | | | |
| First hour | | | 104 | 93 | 97 |
| Second hour | | | 90 | 83 | 82 |
| Third hour | | | 77 | 70 | 73 |
| Fourth hour | | | 67 | 64 | 59 |
| Total | | | 338 | 310 | 311 |
| $Q^{N_{2_A}} = X^{N_{2_A}}$/milligrams dry weight /hour, cubic millimeters: | | | | | |
| First hour | | | 17.4 | 15.5 | 15.7 |
| Second hour | | | 15.1 | 13.9 | 13.3 |
| Third hour | | | 12.8 | 11.6 | 11.8 |
| Fourth hour | | | 11.1 | 10.7 | 9.4 |
| Average | | | 14.1 | 12.9 | 12.6 |
| Chemical data (lactic acid): | | | | | |
| Colorimetric readings: | | | | | |
| $R_1$ | | 103 | 317 | 276 | 288 |
| $R_2$ | | 100 | 322 | 288 | 300 |
| Average, $R = (R_1 + R_2)/2$ | | 101 | 319 | 282 | 294 |
| R−B (F = 40, B = 18) | | 83 | 293 | 264 | 276 |
| T, total LA, cubic millimeters/vessel | | 134 | 474 | 428 | 447 |
| N, net LA, cubic millimeters/vessel | | | 340 | 294 | 313 |
| $Q^{N_{2_{LA}}} = N$/milligrams dry weight/hour | cubic millimeters | | 14.2 | 12.2 | 12.7 |

[1] See table 1, methylcholanthrene-induced tumor 16, No. 9793, June 3, 1940; 37.5°; Ringer-bicarbonate-glucose medium; percent dry weight (initial) = 14.0 mg. dry weight/100 mg. wet weight of aliquot tissue, or 14.0 percent. Vessels with tissue were placed on bath 4¼ hours after animal was killed; equilibrated, and read for preliminary 15-minute period (in three here unrecorded 5-minute intervals, in fact). Immediately after readings taken at $t$ = 0, aerobic manometer stopcocks were turned to position 2 (shutting off No. 1 vessels from external atmospheric pressure), and acid was tipped into control vessels to kill tissue: aerobic, No. 1 vessels; anaerobic, vessel 35. Experimental periods were terminated by tipping of acid: aerobic, 2 hours, No. 2 vessels; anaerobic, 4 hours, vessels 33, 34, and 36. Acid tipping is indicated by underscoring, as is also alkali admission in aerobic No. 1 and 2 vessels. Aerobic No. 2 vessels readings in between $t$ = 0 and final $t$ = 120 minutes were not strictly necessary but were taken as incidental time course observations to note constancy of readings, or otherwise; actual pressure changes per given time interval and corrected for T. B. are not reported. Aerobic vessel No. 1 readings were not strictly necessary but were taken to observe, in arms closed against atmospheric pressure, manometer fluid rises, which should be approximately constant in all vessels with a given initial bicarbonate addition.

[2] Thermobarometer, simply Warburg manometer containing medium but no tissue, otherwise set up and equilibrated identically with other manometers.

[3] $k_{gas} = [(V_{gas} \cdot 273/T° + V_{liquid}\ \alpha)]/P_o$ square millimeters, where $V_{gas}$ and $V_{liquid}$ are cubic millimeters of gas phase and of total liquid phase, respectively; $P_o$ is (for Brodie's solution manometer confining fluid) 10,000 mm.; and $\alpha$ is the Bunsen absorption coefficient (volume of gas concerned reduced to 273° A. and 760 mm. Hg absorbed by 1 volume of medium when partial pressure of gas is 760 mm.); $k_{gas}$ as defined is independent of external pressure.

[4] In converting millimeters $h_{gas}$ to cubic millimeters $X_{gas}$, $X_{CO_2} = +(h_1 - h_2)\ k_{CO_2}$ cubic millimeters of carbon dioxide are produced, but $X_{O_2} = -h_2 k_{O_2}$ oxygen consumed, etc., where $(h_1 - h_2)$ and $h_2$ are the respective partial pressure changes caused by the gases concerned, in millimeters of confining fluid. For gases which disappear, with decrease in pressure ($h_{gas}$ negative in value), it is usually best to consider the corresponding cubic millimeters of gas as consumed (rather than produced), so that a negative sign is applied to the expression $h_{gas}k_{gas}$; one thus has a positive consumption, rather than a negative production.

[5] 1 cm.$^3$ of liquid removed from vessel at end of experiment, diluted 1:10 with $H_2O$, 1 cm.$^3$ of this diluted 1:10 further (D = total dilution = 100) during deproteinization (by copper-lime treatment) and clarification; 1 cm.$^3$ of this analyzed by method of Barker and Summerson (27) against standards $S_1$ and $S_2$ containing $2\gamma$ and $5\gamma$ lactic acid (prepared from dried zinc, lithium, or calcium lactate). $T$, total lactic acid, as mm.$^3$ vessel, is $(R - B)\ DV_{liq}/CFV_{aliquot}$, where $R$ is the average colorimetric reading of the unknown, $D$ is the dilution factor, $C$ is the conversion factor for $\gamma$ to mm.$^3$ lactic acid (=4), $V_{liq}$ is the total liquid volume containing the lactic acid (after any mixing of medium, HCl, etc.), $V_{aliquot}$ is the aliquot of $V_{liq}$ taken, $F = (R_{S_2} - R_{S_1})/(5 - 2) =$ colorimetric reading per $\gamma$ lactic acid, and $B = (R_{S_2} - 5F)$ [should = $(R_{S_1} - 2F)$] = blank reading due to reagents.

### Slicing

Tumor tissue from a freshly killed animal was kept moist with Ringer's solution, and thin free-hand slices, obtained with a single-edged safety razor blade or a Terry slicer (*29*), were stored in a shallow, unaerated reservoir of sugar-bicarbonate-free Ringer's solution at room temperature or lower. Upon eventual removal of the slices from the reservoir, adhering liquid was eliminated by gentle blotting and the tissue wet weight obtained by weighing on a torsion balance as rapidly as convenient the desired amount to be placed in each vessel (aerobic usually 100 to 200 mg., and anaerobic 30 to 75 mg.). Caution was taken to prevent displacement or spattering of any medium or wetting of the forceps while putting the tissue into the vessels. To obtain initial dry weights, aliquot portions of the moist tissue were weighed out at the same time and dried at 85° to 95° C. to constant weight.

### Preparation and Pipetting of Medium

The Ringer-bicarbonate-glucose medium employed in the manometer vessels was freshly prepared by mixing 100 cc. of a stock solution of Warburg-Okamoto Ringer's solution (1,000 parts 0.9 percent NaCl, 20 parts 1.15 percent KCl, 20 parts 1.22 percent $CaCl_2$), 20 cc. 0.15 M $NaHCO_3$, and 2.4 cc. 10 percent glucose solution. This medium was equilibrated for 15 to 45 minutes with 5 percent $CO_2$—95 percent $O_2$ (or for anaerobic experiments sometimes 5 percent $CO_2$—95 percent $N_2$ was employed). Extreme care was taken in pipetting equal amounts of the equilibrated clear medium into any given pair of differential aerobic vessels (e. g., 1.00, 2.00, or 3.00 cc.), since the accuracy of the Summerson differential method is markedly affected by a difference of even a fraction of a drop of medium between the control and the experimental vessel (maximum tolerance, 1/100 cc. =ca. 4 $mm.^3$). Although an Ostwald pipette with ground tip to hold a hypodermic needle was highly satisfactory in pipetting the medium into the aerobic vessels, it was found more convenient generally to use an ordinary drain pipette which was finally touched to the inserted liquid so that capillarity would act to retain an exactly or sufficiently equal amount of liquid in the pipette after each aerobic vessel discharge. Before each discharge the tip of the pipette was wiped with a cellulose tissue paper to remove externally adhering medium. No such care was needed with the anaerobic vessels, in which the tolerance was manyfold greater.

To hasten the eventual absorption of $CO_2$ by alkali, for the final $h_2$ reading a small roll of Whatman (e. g., No. 40) starch-free filter paper, made from a piece about 1 inch square, was inserted in the center well of the aerobic vessels, thereby increasing the absorbing surface upon admission of the 10 percent KOH from the well by turning the stopcock underneath. The vessels were tightly fitted onto their respective manometers by turning them back and forth with gentle pressure until they stuck, that is, no longer turned.

### Equilibration

#### *Aerobic Vessels*

A given series of aerobic vessels were equilibrated together, with a manifold and *on* the bath, in view of the close duplication of paired vessels required. With manometer T-cock in position 1 (both inner arms open to atmospheric pressure) and sidearm vents open, 5 percent $CO_2$—95 percent $O_2$ was passed through the manometers for 10–15 minutes, with a pressure head of about 100-

mm. Brodie solution, care being taken in any event to keep equal pressure heads (within 5 mm.) in any pair of differential vessels. During this interval the manometers were shaken, but the shaking was stopped at the end of the 15-minute period, the gas turned off at the tank, and the vessel vents were closed *simultaneously* in any given differential pair. With the T-cock still in position 1, the manometer fluids were adjusted to approximately the calibrated constant-volume mark on the outer limbs. After equilibration to atmospheric pressure by further shaking for 1 to 2 minutes, the manometer fluids were brought exactly to the calibration mark and the vessels were shut off from the air by closing left and right stopcocks *simultaneously*. The first reading was then taken on the inner limbs, the outer limbs being set at the constant-volume calibration mark, and the manifold was removed at convenience. The tissues in both the No. 1 and No. 2 vessels were allowed to metabolize for an optional preliminary observational period of 0 to 15 minutes before the start of the readings for the actual experimental period. The thermobarometer (T. B.) was read together with the experimental vessels.

At the termination of this preliminary interval the $t=0$ readings were taken, all inner manometer arms were locked against atmospheric pressure by setting the T-cock in (diagonal) position 4, the manometers were removed from the bath, and the acid was tipped into the No. 1 control vessels. The manometers were put back into the bath immediately, and the T-cocks were turned to position 2 (control vessel inner arms shut off from, and experimental vessel inner arms open to, atmospheric pressure). Time-course observations of the total metabolism pressure changes caused by the tissues in the No. 2 vessels were followed for 2 hours at (optionally) 15-minute intervals, the readings being corrected for thermobarometric changes. The total reading of the experimental period is the $h$ reading, a composite figure that is the result of aerobic glycolysis, carbon dioxide production, and oxygen consumption. The Dickens-Greville, rather than the Dixon-Keilin method of obtaining $h$ readings was followed, although either method could be employed, even simultaneously (cf. Summerson (*26*)).

At the termination of the experimental period, the procedure followed for the tipping of the acid in the No. 1 vessels was followed for the No. 2 vessels, the T-cock being left, however, at position 4 for a few minutes and then turned, not to position 2, but to position 3 (differential, pressure in vessel 1 opposing that in vessel 2).

The $h_1$ reading represents the sum of the pressure changes due to oxygen consumption and carbon dioxide production. When constancy of differential readings was attained (approximately 20 to 40 minutes), the $h_1$ readings were calculated for the differential pressure change between the readings at the beginning of the experimental period ($t=0$) and the final equilibrated differential readings. The $h_2$ reading represents the oxygen consumption alone and was obtained from the final differential reading (calculated to $t=0$) after admission from the bottom vessel stopcock of the alkali to absorb the $CO_2$. Following completion of the $h_2$ reading, the vessel stopcocks were opened to the atmosphere simultaneously, the T-cocks opened to position 1, the manometers removed from the bath, and aliquot portions (generally 1 cc.) of the medium removed at convenience for lactic acid or other chemical analysis.

### *Anaerobic Vessels*

The anaerobic series were generally equilibrated *off* the bath at room tem-

perature for 15 to 30 minutes by passing 5 percent $CO_2$—95 percent $N_2$ through a heated tube of reduced copper oxide particles or copper mesh (to remove any traces of $O_2$) and thence to a series of manometers and vessels by means of a manifold. A pressure head of about 100 mm. was maintained during equilibration, and all vents were checked for the free passage of gas to insure complete displacement of air and saturation with $CO_2/N_2$. At the conclusion of the equilibration period the vessel vents and manometer T-cocks were closed, and the manometers were placed on the bath and shaken. Acid was tipped into the lactic acid control vessels at $t=0$ to kill the tissue. Before this was done, however, the brom thymol blue was first tipped into the medium and the pH observed colorimetrically with standards. Readings were taken on the experimental vessels every 15 minutes for the first hour, and thereafter half hourly for generally 3 additional hours. At the conclusion of the experimental period, brom thymol blue and acid were tipped into all remaining vessels, the manometers with T-cocks opened were removed from the bath, and aliquot samples (ordinarily 1 cc.) for lactic acid determinations were removed at convenience (up to 24 hours).

## TUMOR MATERIAL

The following transmissible fowl tumors, all sarcomas, were employed in this investigation: chicken tumors 11 (*24*), 12 (*23*), 13 (*22*), and 16 (Furth and Kabat, unpublished data), obtained from and originating with the Department of Pathology, Cornell University Medical College; and chicken tumors 10 of Murphy and Claude (yet to be described), and 1 of Rous (*30*) (Nos. 2549–2553), obtained from and originating with the Rockefeller Institute for Medical Research. Tumor 1, Nos. 10284, 10285, 10287, and 20288, was cultured in the Department of Pathology from a stock culture obtained from Dr. David Shemin, College of Physicians and Surgeons. Tumor 1, Nos. O, P, Q, and R, grown on the chorioallantoic membrane of chick embryos, was also studied.

Material for metabolic study was obtained by serial passage of the tumor in the breast and leg muscles of healthy chicks (2 days to 1 month old at the time of inoculation). In the case of tumor 12, the tumor particles were injected into the sternum and the wing. Tumors were excised about 3 to 5 weeks after injection.

The tumors were carefully dissected and freed from macroscopically observable muscle for the metabolism studies, and representative aliquot sections were fixed with Bouin's solution, cut, and stained with haematoxylin and eosin. Ordinarily it was not feasible to use the identical tissue slices metabolized for sectioning and staining, since the metabolized slices would usually have been acidified to about pH 1 during the determinations. In spite of careful dissection, the microscopic sections often showed conspicuous amounts of muscle, and areas of degeneration, necrosis, and leukocytic infiltration, as will be discussed more fully later. We feel satisfied, nevertheless, that the material sectioned was adequately representative of the slices metabolized, as regards aliquots and from a statistical viewpoint.

## RESULTS

Before considering the metabolism results in detail, brief characterizations will be given of the tumor materials actually employed, as observed in the histologic examinations. The latter, from the standpoint of the metabolic studies, were intended to answer questions that are ordinarily given little consideration in the course of routine macroscopic examinations. Since the metabolic values are ex-

pressed in terms of unit weight of tissue, it is of much importance to know roughly what proportion of the material metabolized is composed of living malignant cells and what is the remaining material that will be referred to in this paper as impurity. The designation of cells as living or viable on the basis of their appearance in fixed and stained preparations is obviously arbitrary. Tumor cells with well-preserved nuclear structure are conventionally regarded as viable, whereas those with pyknotic or fragmented nuclei are considered nonviable or dead. It is noteworthy, however, that changes in nuclear structure may not set in until several hours after death.

The impurities here considered are of two kinds: (1) Intercellular and histologically dead tissue; and (2) metabolically active impurities as follows: (a) living, nonmalignant cells that like stroma and blood vessels are integral parts of the growth, (b) invaded host tissue, most frequently muscle, and (c) leukocytes of different sorts which have invaded the tumor. From the standpoint of histologists, the stroma cells are an integral part of the growth; but from the standpoint of metabolism, they are more conveniently classified among the impurities because the aim is in part to characterize the tumor cells per se and the presence of variable, often large numbers of nonmalignant stroma cells may, if not considered separately, obscure the metabolic characterization attempted. It is possible, of course, that these cells, as well as invading leukocytes, may alter or modify the metabolism of the tumor cells themselves, although we have obtained little suggestive evidence for this.

The foregoing considerations, which arose during the course of the investigation, rendered it desirable to present illustrations of tumors even already known and well-described, but showing, however, mainly the impurities frequently encountered, rather than the pure tumors. The various photomicrographs are provided to focus the attention of those engaged in similar studies on the complexity of the problem of defining tumor metabolism when tissue slices of the usual size are employed. We have attempted to give, with each tumor, one or two illustrations of aliquots of the typical tumor tissue metabolized, together with examples of prominent nontumor cells histologically included in the slices even upon most careful gross dissection. For this purpose, it has been deemed preferable to give a composite of several higher power fields rather than a single low-power field, although the latter would be desirable too. We do not attempt to give an accurate quantitative representation of the proportions of all of the different types of cells found in any particular sample metabolized, but in the following descriptions we shall endeavor to indicate an average picture for each set of tumor materials that should be of considerable value in attempting to analyze the results of the metabolic studies. The estimates will be based not only on the present histologic and photomicrographic studies but also on past experience with these chicken tumors, covering a good many years of observation and published descriptions.

## Microscopic Appearance of Tumors Metabolized

### *Methylcholanthrene-induced Chicken Tumor 16 of Furth and Kabat*

Methylcholanthrene - induced chicken tumor 16 (fig. 1) is derived from a tumor induced by intramuscular injection of methycholanthrene and transmitted by cell grafts to healthy fowls. Numerous attempts to demonstrate a filterable agent, by using very sensitive tests, have been

unsuccessful. The tumor is composed of spindle-shaped cells that produce comparatively little or no mucinous or fibrous intercellular substance. The cells are more closely packed than those of any other tumors studied in this investigation. There are usually many mitotic figures. The tumors employed contained relatively small amounts of neighboring host tissue or necrotic tumor and were therefore exceptionally easy to handle in the metabolism experiments. The differences in the metabolism between different samples of this tumor were not great, and the microscopic examination of the sections could seldom be expected to yield information concerning the possible cause of these differences.

*Chicken Tumor 10 of Murphy and Claude*

Chicken tumor 10 of Murphy and Claude (fig. 2) arose about 14 years ago at approximately the site of an injection of chicken tumor 9 of Murphy and Landsteiner (*14*), a transplantable, spindle-cell sarcoma that had had its origin in a tumor induced by repeated injections of coal tar. Tumor 9, as such, is now extinct. It could not be transmitted by a cell-free agent, and neither has tumor 10 been filterable until recent years, at least not without removal of natural inhibitor (*31*).

Tumor 10 is composed of fibroblastlike cells of fairly uniform size and shape and staining properties. Mitotic figures are abundant, and the tumor is highly invasive, although the cells are not conspicuously hyperchromatic. The stroma is scarce as a rule, and in many places the tumor cells are widely separated by spaces containing faintly staining, "foamy" intercellular substances (fig. 2, *C*) presumably rich in polysaccharide. The tumor contains few blood vessels, and it is nearly free from necrosis.

The metabolic values on samples of this tumor were again fairly uniform, and the average anaerobic glycolysis values were in fact the highest for any tumor examined. The lowest individual sample (table 1, No. 2544; fig. 2, *C* and *D*) had areas of necrosis with large numbers of monocytes and a few lymphocytes and polymorphonuclear leukocytes; the sample with the highest anaerobic metabolism (table 1, No. 2543; fig. 2, *A*) showed in most microscopic fields almost pure tumor tissue, although in many areas there were diffuse infiltrations of polymorphonuclear leukocytes (fig. 2, *B*).

FIGURE 1.—Chicken tumor 16, a transmitted methylcholanthrene - induced sarcoma (No. 9594). There is a dense growth of spindle-shaped cells and several mitotic figures. × 450.

*Chicken Tumor 11 of Furth*

The malignant cells of chicken tumor 11 (fig. 3) are spindle-shaped, or polymorphous, or large mononuclear cells, similar

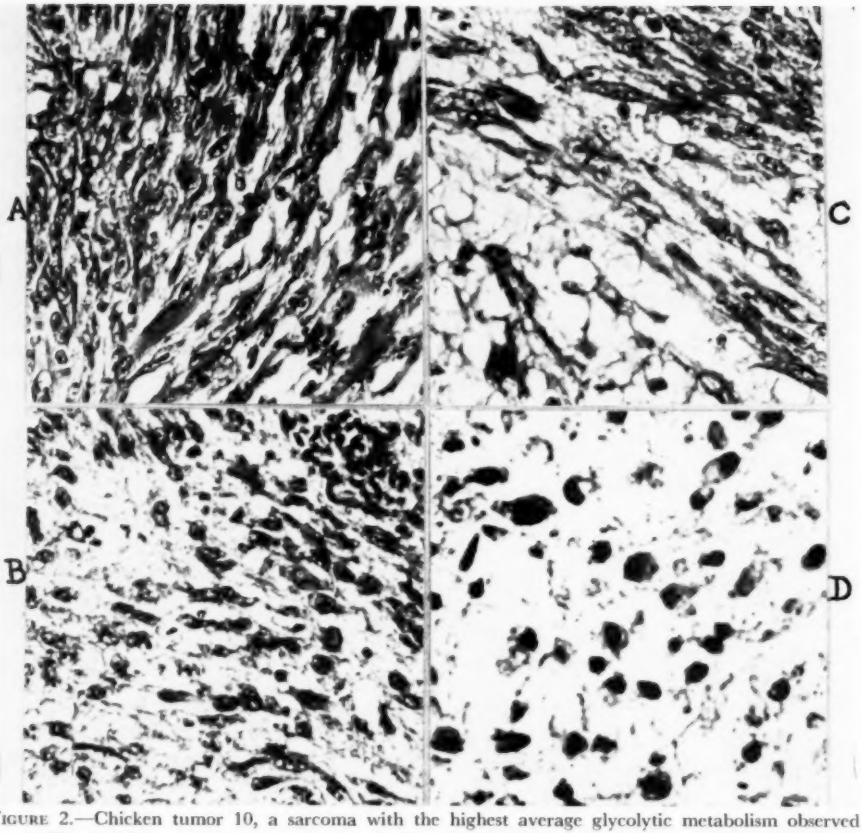


FIGURE 2.—Chicken tumor 10, a sarcoma with the highest average glycolytic metabolism observed. *A* (No. 2543), a relatively pure tumor; *B* (No. 2543), infiltrations by many polymorphonuclear leukocytes; *C* (No. 2544), intercellular "foamy" substance, presumably polysaccharide, among well-preserved tumor cells; *D* (No. 2544), degenerated part of tumor with many monocytes and a few other leukocytes and with congested blood vessels. × 450.

Fig. 2 & 5 prints reversed

to those of chicken tumor 1 of Rous. The tumor is slow-growing and tends to become fibrous and infiltrated with lymphocytes. The texture of the tumor varies greatly. In some fields the tumor cells are fairly densely packed, in others rather loosely. On the whole this tumor is less pure than either tumor 10 or tumor 16, although metabolically considered it was almost identical with the latter, except for somewhat greater variability. It was not possible to make any consistently close correlation between metabolism of individual samples and microscopic appearance on a basis of extent of lymphoid infiltration, hemorrhage, or necrosis. Figure 3, *A* (No. 10045), shows a characteristic field of a relatively pure tumor tissue that had about the highest anaerobic glycolysis observed with this strain (table 1, tumor 11). Figure 3, *B* and *C* (No. 9967), show advanced lymphoid infiltration, which was widespread in this tumor in which aerobic glycolysis was relatively

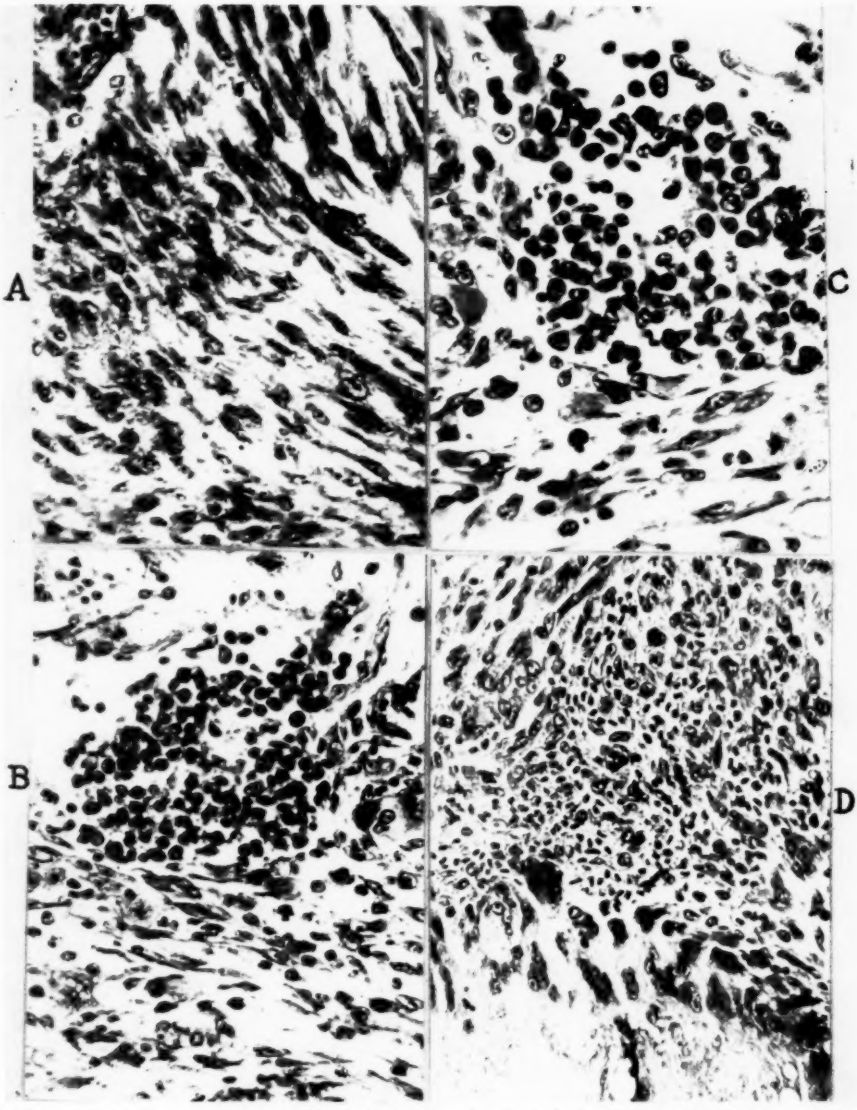


FIGURE 3.—Chicken tumor 11. *A* (No. 10045), example of relatively pure portion of tumor. × 450; *B* and *C* (No. 9967), loose growth with several mitotic figures, advanced lymphoid infiltration, and remnants of muscle fibers. *B*, × 450; *C* × 750; *D* (No. 9950), margin of necrotic area with multinucleated giant cells and an area of hemorrhage adjacent to tumor. × 450.

low. In one tumor (No. 9950, fig. 3, *D*) there were numerous large areas of necrosis of undetermined character surrounded by giant cells similar to those of avian tubercles. Occasional areas of hemorrhage were seen about these necrotic areas. Similar lesions were not seen in any of the other tumors studied.

### *Chicken Tumor 12 of Furth*

Chicken tumor 12 (fig. 4), a slowly growing osteochondrosarcoma, occurs with or without accompanying leukosis. The malignant cells are osteochondroblasts that may proliferate with or without bony or cartilaginous matrices. In the present series used for metabolizing, neither leukosis nor bone formation was observed, and the material metabolized consisted either of almost pure masses of the malignant osteochondroblasts (fig. 4, *A* and *B*) or of malignant cartilaginous tissues with varying amounts of intercellular substance (fig. 4, *C* and *D*). As pointed out in the original paper describing this growth (*23*), the osteochondrogenic character of these large round cells is not evident when the cells are present in pure culture (fig. 4, *A* and *B*). However, all stages of cartilage and bone formation can be seen in various preparations, particularly in the more slowly growing tumors, indicating the potentiality to form bone. The cells are larger than any primitive blood cells, which they resemble in shape and general outline, but are characterized by a clear nucleus that seems to contain nothing but a large darkly staining nucleolus. The recent phosphatase studies of Kabat and Furth (*32*) included numerous tumor samples used in the present metabolism studies. Neither the usual sarcoma nor the leukosis cells contained phosphatase, whereas the pure osteochondrosarcoma cells reported upon here contained large amounts of this enzyme, even in the complete absence of cartilage or bone. The osteochondrogenic character of these large round cells thus does not require the unfailing appearance of osteoid tissue or cartilaginous matrix.

In general, a fair negative correlation could be observed between the extent of glycolytic metabolism and the amount of cartilage formation. Sample No. 10049 (fig. 4, *C* and *D*) had the least such metabolism and the most cartilage, whereas No. 9943 (fig. 4, *A*) had the highest glycolysis and almost no cartilage; No. 9947 (fig. 4, *B*, *E* and *F*) had intermediate glycolysis and a moderate amount of varying impurity (table 1, tumor 12). The low average rate of glycolysis of tumor 12 parallels the expectation based on the known very low rate of metabolism of cartilaginous normal tissue (*33*, *34*).

### *Chicken Tumor 13 of Stubbs and Furth*

Chicken tumor 13 (fig. 5) is a sarcoma that was produced by intramuscular injection of a filterable agent which upon intravenous injection may also produce erythroleukosis. The tumors usually contained large and variable quantities of mucinous intercellular material that has been shown to be of a polysaccharide nature (*35*). The cells resemble the usual spindle-cell sarcoma, and occasionally contain multinucleated giant cells. On the whole, these tumors are relatively impure, and the largest amount of impurity is the intercellular substance rich in polysaccharide material. Leukocytic infiltration is very common. Necrosis was slight except in No. 9527. The great degree of histologic variability and impurity made detailed metabolic correlation difficult without a very considerable amount of sectioning, but there was correspondingly great variability among metabolism replicates (table 1, tumor 13).

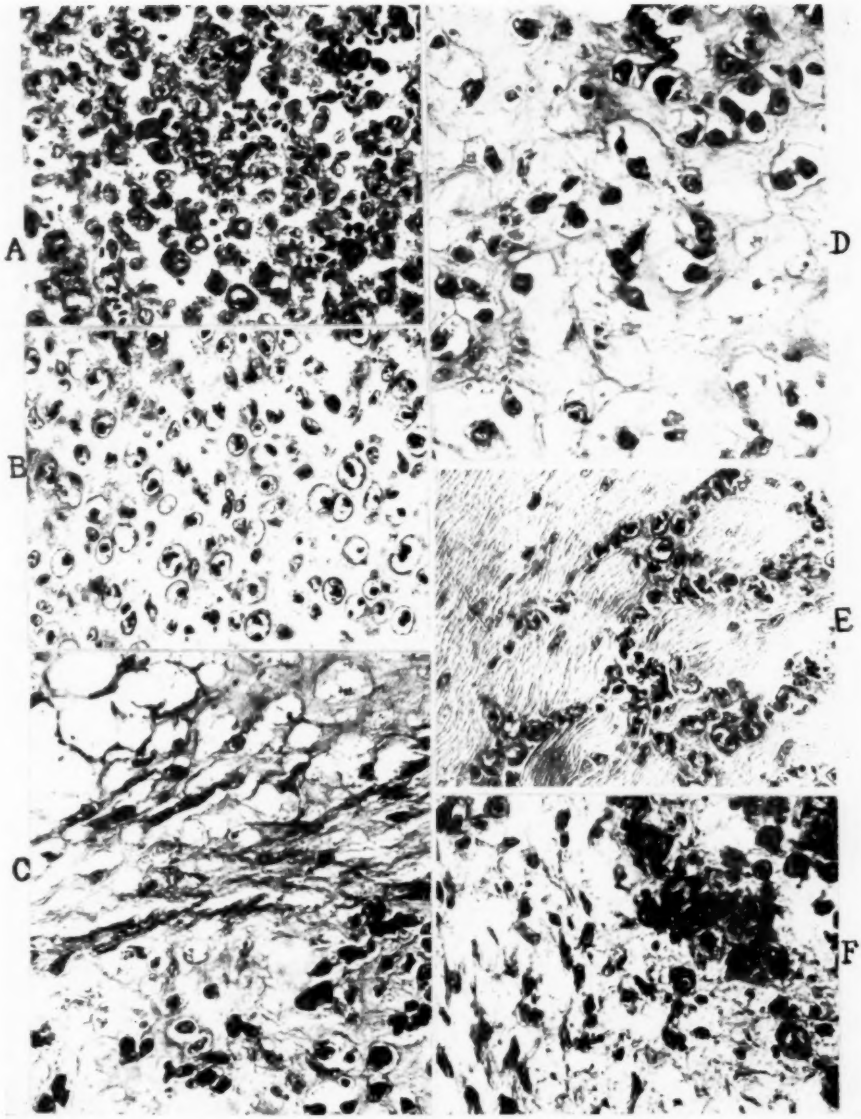


FIGURE 4.—Chicken tumor 12. *A* (No. 9943), the large round cells are osteochondroblasts with scattered erythrocytes. × 450; *B* (No. 9947), densely packed malignant osteochondroblasts with almost no other cells. The cells resemble cartilage cells, but intercellular substance is absent. × 700; *C* (No. 10049), cartilage with degenerating cartilage in the tumor. × 450; *D* (No. 10049), cartilage cells with large amounts of cartilaginous matrix. × 450; *E* (No. 9947), invasion of muscle by malignant osteochondroblasts. × 450; *F* (No. 9947), malignant osteochondroblasts (large, round cells) with spindle-shaped cells that are probably not malignant, and edematous stroma. × 450.

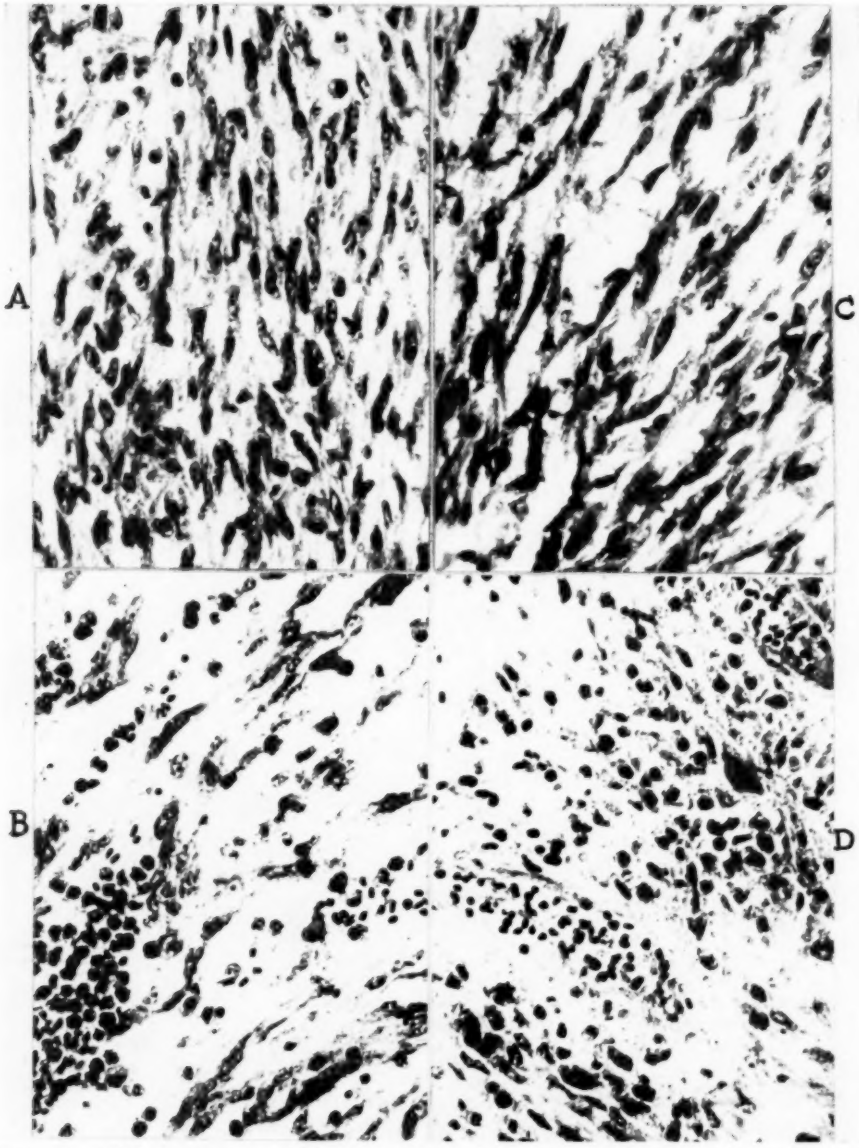


FIGURE 5.—Chicken tumor 13.—*A* (No. 9853), characteristic tumor cells of the strain infiltrating muscle tissue. ×450; *B* and *C* (No. 9690), tumor with slight and moderate amounts of mucinous stroma, respectively. ×450; *D*, (No. 9690) area with degenerated tumor cells and monocytes. ×750.

Fig. 2 & 5 prints reversed

*Chicken Tumor 1 of Rous*

Chicken tumor 1 of Rous (fig. 6), the first sarcoma transmitted by a filterable agent, contained the usual spindle-shaped and polymorphous cells, with variable amounts of intercellular substance. There were often large numbers of monocytes in the tumor, whose effects on the observed metabolic values cannot as yet be assessed since there are no data on their metabolism. It is noteworthy that sample No. 10287 (fig. 6, *A* and *C*), which exhibited the highest glycolysis (table 1, tumor 1 of Rous), contained large numbers of monocytes.

This tumor was also cultivated, following the method introduced by Murphy (*36*), on the chorioallantoic membrane of the developing chick embryo and was used for metabolic studies 5 days after inoculation of 0.1 cc. fresh tumor pulp into 12-day incubated chicken eggs. The embryo-cultured tumors were of two major gross types: nodular, opaque, and ivory colored; and membranous, transparent, slightly yellow in color, and like firm gelatin in consistency; several samples contained considerable amounts of blood. The gross tumors designated as nodular (fig. 7, *A*, *B*, and *C*) proved on microscopic examination to be composed of tumor cells, with many mitotic figures, only very few leukocytes, and blood vessels containing some erythrocytes. The bloody material proved to be rich in congested capillaries (with rather slight hemorrhage) and contained a considerable number of polymorphonuclear leukocytes. The membranous material was found to be heavily infiltrated with mature polymorphonuclear leukocytes, and with some maturing myelocytes; tumor cells were doubtless very few, and it is uncertain how many there actually were because of the difficulty of distinguishing them from fibroblastlike embryonal cells. There were numerous mitotic figures, but many of these were in the embryonal cells. Good metabolic correlation with tumor histology was possible (table 1, tumor 1 cultured on egg embryo) in view of the striking difference between the nodular and membranous type (fig. 7).

METABOLIC DATA

The metabolic data are presented in the protocol and in tables 1–4. Standardized sets of metabolic data for each tumor are given in table 1. In this table the experiments are arranged in the order of their dates of occurrence, to indicate the sequence of the transplants involved and the order in which we acquired experience both with any given tumor and with different tumors. The replicate determinations, aerobic and anaerobic, are listed in decreasing order of the glycolysis values. It should be clearly understood that there is no necessary relationship between the number of aerobic and anaerobic replications; and in particular, that there is no connection between a given row of anaerobic data on the same line with a set of aerobic data, since the aerobic and anaerobic data were always obtained in different vessels, and in no instance with the same particular pieces of tissue. Chemical $Q_{LA}$ and manometric $Q_A$ values and their ratios on a given row correspond, of course.

*Aerobic Q Values*

It is our feeling that differences between replicates are accounted for about equally on the bases of (1) actual differences in the aliquot portions of sliced material (wet weight/dry weight ratios, percentages of any connective tissue, muscle, necrosis, etc.), and (2) manipulative errors. In a survey of the magnitude of this investigation, wherein large metabolic differences

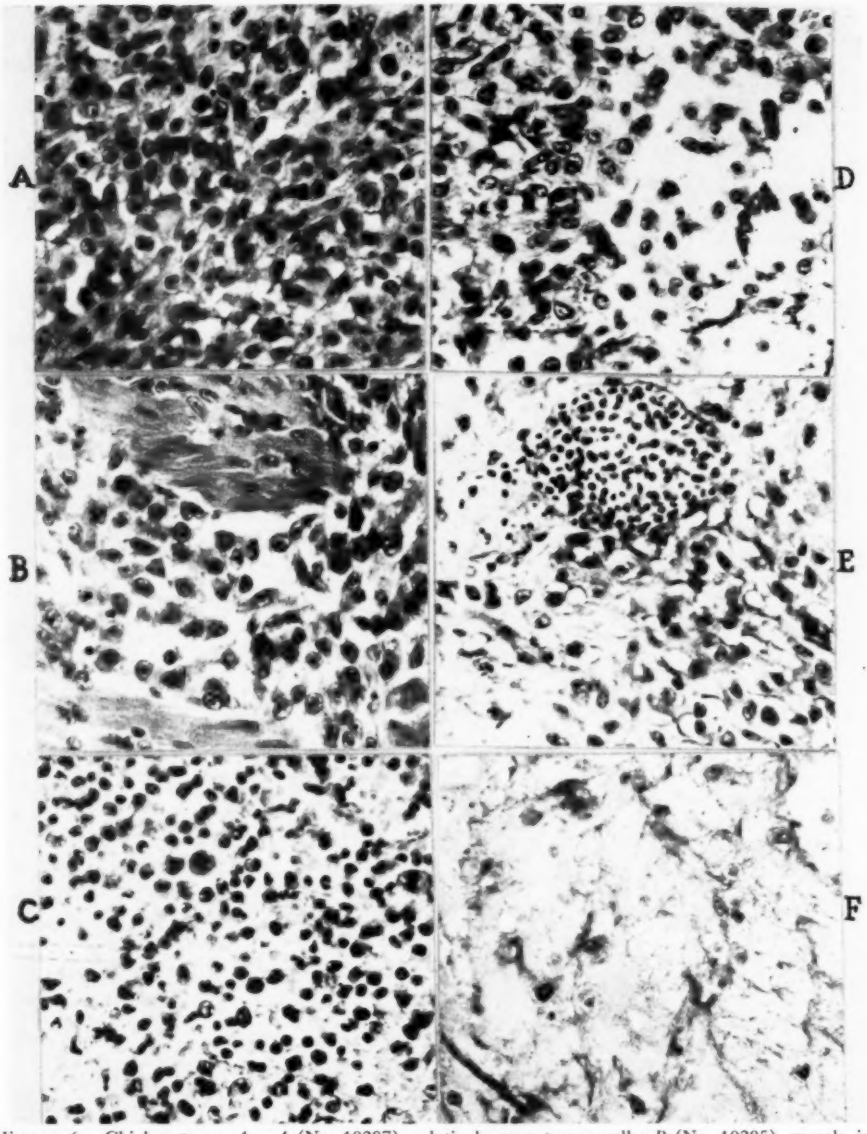


FIGURE 6.—Chicken tumor 1.—*A* (No. 10287), relatively pure tumor cells; *B* (No. 10285), muscle invaded by tumor; *C* (No. 10287), only an occasional cell resembling a tumor cell is seen among masses of mononuclear cells, most of which appear to be monocytes, with some lymphocytes; *D*, *E*, and *F* (No. 10288), sarcoma cells with varying amounts of intercellular substance: in *D*, small amount, with accumulation of mucinous material; in *E*, moderate amount, with vacuolization and degeneration of tumor cells and an enormously dilated capillary filled with erythrocytes; in *F*, enormous amount, with few tumor cells. ×450.

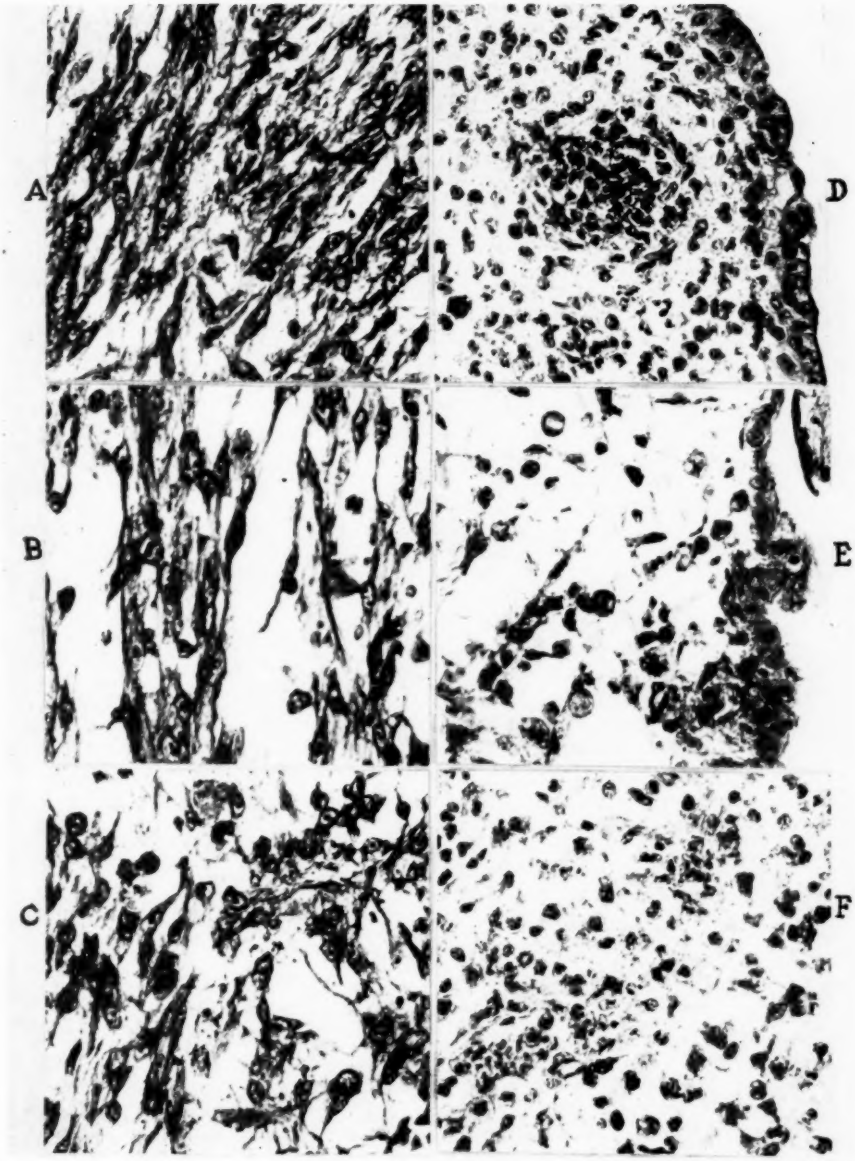


FIGURE 7.—Chicken tumor 1 grown on chorioallantoic membrane of chick embryo. *A*, *B*, and *C* (No. Q), from nodular growth with a relatively pure culture of tumor cells; *D*, *E*, and *F* (No. R), from membranous growth, with occasional tumor cells in the embryonal mesenchyme, which is densely infiltrated with white blood cells of various types. Thickened epithelial cells of the membrane are seen on the right margins of *D* and *E*. × 450.

425485—41——2

TABLE 1.—*Standard metabolic determinations of chicken tumors*

METHYLCHOLANTHRENE-INDUCED CHICKEN TUMOR 16

| Chicken No. | Date | Temperature | Dry weight | Determinations: Manometric: Aerobic: $Q^{O_2}_A$ | $Q_{CO_2}$ | $Q_{O_2}$ | R. Q. | Anaerobic: $Q^{N_2}_A$: Hour: 1st | 2d | 3d | 4th | Average | Chemical: $Q^{N_2}_{LA}$ | $Q^{O_2}_{LA}$ | Chemical/Manometric: $Q^{N_2}_{LA}/Q^{N_2}_A$ | $Q^{O_2}_{LA}/Q^{O_2}_A$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *1940* | *° C.* | *Pct.* | | | | | | | | | | | | | |
| 9581 | Mar. 29 | 37.5 | 14.8 | 10.6 | 4.8 | 4.7 | 1.02 | 15.9 | 16.4 | 14.9 | ..... | 15.7 | 14.1 | 12.4 | 0.90 | 1.17 |
| | | | | 8.8 | 6.7 | 5.9 | 1.14 | 15.0 | 16.0 | 14.9 | ..... | 15.3 | 12.4 | 6.4 | .81 | .74 |
| | | | | 7.2 | 4.8 | 5.4 | .89 | 14.4 | 16.8 | 14.9 | ..... | 15.4 | 12.4 | 6.4 | .81 | .89 |
| | | | | ..... | ..... | ..... | ..... | 16.7 | 14.1 | 13.2 | [1]12.6 | 14.1 | 11.3 | ..... | .80 | ..... |
| | | | | 7.2 | 4.5 | 6.1 | .74 | 16.2 | 14.3 | 12.1 | [1]12.6 | 13.8 | 12.2 | 5.2 | .88 | .72 |
| | | | | 5.9 | 3.6 | 5.3 | .68 | 15.9 | 13.6 | 12.2 | 12.2 | 13.5 | 12.1 | 6.0 | .90 | 1.02 |
| | | | | 5.9 | 4.1 | 4.7 | .87 | 13.2 | 11.3 | 11.5 | [1]11.0 | 11.7 | 8.2 | 4.2 | .70 | .71 |
| 9595 | Apr. 8 | 37.0 | 15.0 | 6.5 | 3.4 | 2.8 | 1.21 | 11.5 | 11.5 | 9.8 | ..... | 10.9 | ..... | ..... | ..... | ..... |
| | | | | 6.0 | 3.1 | 3.2 | .97 | 7.9 | 7.0 | 7.2 | ..... | 7.4 | ..... | ..... | ..... | ..... |
| | | 41.0 | ..... | [2]6.2 | [2]5.9 | [2]5.0 | [2]1.18 | [2]12.6 | [2]13.1 | [2]11.3 | ..... | [2]12.3 | ..... | ..... | ..... | ..... |
| | | | | [2]4.8 | [2]5.8 | [2]6.7 | [2].87 | [2]10.9 | [2]10.0 | [2]9.3 | ..... | [2]10.1 | ..... | ..... | ..... | ..... |
| | | | | ..... | ..... | ..... | ..... | [2]9.7 | [2]9.7 | [2]9.2 | ..... | [2]9.5 | ..... | ..... | ..... | ..... |
| 9594 | Apr. 12 | 39.0 | 15.0 | 11.8 | 4.9 | 5.2 | .94 | 19.6 | 18.5 | ..... | ..... | 19.0 | ..... | ..... | ..... | ..... |
| | | | | 10.6 | 4.7 | 5.6 | .84 | 20.9 | 18.7 | 16.7 | ..... | 18.8 | ..... | ..... | ..... | ..... |
| | | | | 9.6 | 3.8 | 5.0 | .76 | 19.5 | 17.5 | 16.0 | ..... | 17.7 | ..... | ..... | ..... | ..... |
| 9794 | May 31 | 37.5 | 13.6 | 7.3 | 4.5 | 5.2 | .87 | 16.7 | 13.9 | 11.5 | [1]12.1 | 13.5 | ..... | ..... | ..... | ..... |
| | | | | 6.8 | 3.5 | 4.6 | .76 | 13.9 | 12.9 | 11.5 | [1]11.1 | 12.3 | ..... | ..... | ..... | ..... |
| 9792 | May 31 | 37.5 | 11.7 | 11.0 | 3.8 | 4.6 | .83 | 21.6 | 17.4 | 15.5 | [1]14.3 | 17.2 | ..... | ..... | ..... | ..... |
| | | | | 9.3 | 5.3 | 5.5 | .96 | 20.7 | 17.5 | 16.2 | [1]14.3 | 17.2 | ..... | ..... | ..... | ..... |
| | | | | ..... | ..... | ..... | ..... | 17.9 | 15.8 | 14.2 | [1]13.1 | 15.2 | ..... | ..... | ..... | ..... |
| 9793 | June 3 | 37.5 | 14.0 | 7.3 | 4.1 | 4.7 | .87 | 17.4 | 15.1 | 12.8 | 11.1 | 14.1 | 14.2 | ..... | .99 | ..... |
| | | | | 7.0 | 4.4 | 4.8 | .92 | 15.5 | 13.9 | 11.6 | 10.7 | 12.9 | 12.2 | 6.1 | .95 | .87 |
| | | | | 6.8 | 4.2 | 4.6 | .91 | 15.7 | 13.3 | 11.8 | 9.4 | 12.6 | 12.7 | 5.2 | .99 | .76 |
| | | | | 6.5 | 3.8 | 4.3 | .88 | ..... | ..... | ..... | ..... | ..... | ..... | 5.2 | ..... | .80 |
| 9789 | June 7 | 37.5 | 14.5 | 7.0 | 5.5 | 5.5 | 1.00 | 17.5 | 15.0 | 13.2 | 9.1 | 13.7 | ..... | ..... | ..... | ..... |
| | | | | 6.3 | 5.5 | 4.8 | 1.15 | 15.9 | 13.4 | 12.2 | 8.5 | 12.5 | ..... | ..... | ..... | ..... |
| | | | | 6.0 | 5.8 | 5.3 | 1.09 | 15.8 | 13.2 | 11.4 | 8.3 | 12.2 | ..... | ..... | ..... | ..... |
| 10181 | Oct. 1 | 38.0 | 14.0 | 7.4 | 2.8 | 3.9 | .72 | 16.8 | 11.7 | 11.1 | 11.1 | 12.7 | ..... | ..... | ..... | ..... |
| | | | | 7.4 | 2.8 | 3.8 | .74 | 9.2 | 7.8 | 7.8 | 7.8 | 8.1 | ..... | ..... | ..... | ..... |
| Irradiated [3] | | | | [2]7.9 | [2]2.8 | [2]4.1 | [2].68 | [2]16.5 | [2]13.9 | [2]13.2 | [2]13.2 | [2]14.2 | ..... | ..... | ..... | ..... |
| | | | | [2]7.6 | [2]2.7 | [2]3.8 | [2].71 | [2]10.4 | [2]8.8 | [2]8.8 | [2]8.8 | [2]9.2 | ..... | ..... | ..... | ..... |
| 10271-10275 | Oct. 14 | 38.0 | 15.7 | 6.2 | 2.8 | 2.8 | 1.00 | {17.6 | 16.5 | 14.5 | ..... | 16.2 | ..... | ..... | ..... | ..... |
| | | | | | | | | {11.6 | 12.1 | 10.4 | ..... | 11.4 | ..... | ..... | ..... | ..... |
| Irradiated [3] | | | | [2]6.7 | [2]2.8 | [2]2.7 | [2]1.04 | {[2]14.9 | [2]14.4 | [2]13.0 | ..... | [2]14.1 | ..... | ..... | ..... | ..... |
| | | | | | | | | {[2]11.3 | [2]11.3 | [2]9.8 | ..... | [2]10.8 | ..... | ..... | ..... | ..... |
| (4) | Oct. 15 | 38.6 | 15.7 | {[2]6.0 | [2]2.4 | [2]2.6 | [2].92 | {[2]11.5 | [2]10.3 | [2]10.8 | ..... | [2]10.9 | ..... | ..... | ..... | ..... |
| | | | | {[2]5.9 | [2]1.6 | [2]2.3 | [2].75 | | | | | | | | | |
| Irradiated [3] | | | | {[2]7.2 | [2].47 | [2]1.7 | [2].28 | [2]8.5 | [2]6.9 | [2]7.8 | ..... | [2]7.7 | ..... | ..... | ..... | ..... |
| | | | | {[2]5.0 | [2].36 | [2]1.3 | [2].28 | [2]7.3 | [2]6.4 | [2]6.6 | ..... | [2]6.8 | ..... | ..... | ..... | ..... |
| Grand averages | | 37.7 | 14.4 | 7.7 | 4.3 | 4.7 | .91 | 15.9 | 14.3 | 12.6 | 11.1 | 13.9 | 12.2 | 6.5 | .87 | .85 |
| Determinations, number | | | | 25 | 25 | 25 | 25 | 27 | 27 | 26 | 17 | 27 | 10 | 9 | 10 | 9 |
| Tumors, number | | | | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 6 | 9 | 2 | 2 | 2 | 2 |

See footnotes at end of table.

TABLE 1.—*Standard metabolic determinations of chicken tumors*—Continued

CHICKEN TUMOR 10

| Chicken No. | Date | Temperature | Dry weight | Determinations: Manometric: Aerobic $Q^{O_2}_A$ | Aerobic $Q_{CO_2}$ | Aerobic $Q_{O_2}$ | Aerobic R. Q. | Anaerobic $Q^{N_2}_A$ Hour 1st | 2d | 3d | 4th | Average | Chemical $Q^{N_2}_{LA}$ | Chemical $Q^{O_2}_{LA}$ | Chemical/Manometric $Q^{N_2}_{LA}/Q^{N_2}_A$ | $Q^{O_2}_{LA}/Q^{O_2}_A$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *1940* | *° C.* | *Pct.* | | | | | | | | | | | | | |
| 2544 | Nov. 1 | 38.0 | 8.0 | 15.3 | 2.7 | 5.0 | .54 | 20.2 | 17.7 | 17.7 | 17.7 | 18.2 | 15.3 | 14.4 | .83 | .94 |
| | | | | 10.9 | 2.3 | 4.1 | .56 | 18.5 | 16.7 | 16.7 | 16.2 | 17.0 | 13.3 | 10.7 | .78 | .98 |
| 2548 | Nov. 1 | 38.0 | 10.0 | 12.0 | 4.8 | 5.1 | .87 | 28.5 | 23.4 | 23.3 | 20.5 | 23.9 | 22.6 | 7.6 | .95 | .63 |
| | | | | 10.7 | 4.3 | 4.4 | .97 | 24.4 | 21.9 | 19.7 | 17.8 | 20.9 | 26.9 | 10.1 | 1.29 | .94 |
| 2543 | Nov. 11 | 38.0 | 8.0 | 13.8 | 5.0 | 6.4 | .78 | 29.5 | 28.3 | 28.3 | 26.4 | 28.1 | | | | |
| | | | | 13.7 | 3.2 | 3.6 | .89 | 24.1 | 24.1 | 24.1 | 21.7 | 23.5 | | | | |
| | | | | 13.3 | 5.5 | 5.7 | .97 | 23.2 | 22.4 | 23.6 | 21.0 | 22.5 | | | | |
| Grand averages | | 38.0 | 8.7 | 12.8 | 4.0 | 5.0 | .79 | 24.1 | 22.1 | 21.9 | 20.2 | 22.0 | 19.5 | 10.7 | .96 | .87 |
| Determinations..number | | | | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 4 | 4 | 4 | 4 |
| Tumors.....number | | | | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 2 |

CHICKEN TUMOR 11

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9948 | July 24 | 38.0 | 11.0 | 6.3 | 3.7 | 3.7 | 1.00 | 16.6 | 15.8 | 15.5 | 12.3 | 15.0 | 13.3 | 2.7 | .89 | .43 |
| | | | | 5.7 | 4.2 | 4.2 | 1.00 | 14.7 | 13.3 | 13.6 | 10.5 | 13.0 | 12.9 | 4.3 | .99 | .75 |
| | | | | | | | | 11.9 | 10.9 | 11.3 | 8.1 | 10.5 | 9.5 | | | |
| Unrinsed [4] | | | | 6.4 | 4.1 | 5.4 | .76 | | | | | | | 5.3 | | .83 |
| Preliminary anaerobiosis [5] | | | | 5.7 | 4.9 | 5.5 | .89 | | | | | | | 5.2 | | .91 |
| | | | | 7.8 | 4.3 | 4.9 | .88 | | | | | | | | | |
| | | | | 7.5 | 4.2 | 4.4 | .95 | | | | | | | | | |
| No glucose [7] | | | | [2]6.6 | [2]3.9 | [2]4.8 | [2].81 | | | | | | | [2]5.1 | | [2].77 |
| | | | | [2]6.2 | [2]4.4 | [2]5.2 | [2].85 | | | | | | | [2]3.4 | | [2].55 |
| 9950 | Aug. 2 | 38.0 | 10.0 | 6.4 | 3.7 | 4.5 | .82 | 16.1 | 14.6 | 13.7 | 11.6 | 14.0 | 17.6 | 6.6 | 1.26 | 1.03 |
| | | | | 6.2 | 4.2 | 4.9 | .86 | 15.2 | 11.1 | 13.1 | 9.8 | 12.3 | 13.6 | 5.4 | 1.11 | .87 |
| | | | | 5.2 | 4.7 | 5.5 | .86 | | | | | | | 5.5 | | 1.06 |
| 9969 | Aug. 5 | 38.0 | 9.8 | 8.6 | 5.6 | 6.4 | .88 | 23.4 | 19.8 | 17.5 | 17.5 | 19.5 | 18.6 | 6.2 | .95 | .72 |
| | | | | 8.4 | 6.3 | 6.9 | .91 | 19.4 | 16.8 | 14.9 | 14.9 | 16.5 | 17.2 | 7.5 | 1.04 | .89 |
| | | | | 8.3 | 5.6 | 6.2 | .90 | 18.3 | 16.0 | 14.9 | 14.8 | 16.0 | 16.8 | 10.6 | 1.05 | 1.28 |
| 9971 | Aug. 7 | 37.9 | 9.0 | 8.6 | 6.1 | 7.2 | .85 | 19.4 | 16.7 | 15.3 | 15.3 | 16.7 | | | | |
| | | | | 8.4 | 6.2 | 6.9 | .90 | 19.2 | 15.6 | 14.8 | 14.5 | 16.0 | | | | |
| | | | | 7.5 | 6.1 | 6.5 | .94 | 16.2 | 13.5 | 13.4 | 13.4 | 14.1 | | | | |
| 9967 | Aug. 9 | 37.9 | 10.0 | 6.2 | 6.3 | 6.7 | .94 | 16.9 | 15.7 | 15.1 | 14.7 | 15.6 | | | | |
| | | | | 6.1 | 5.7 | 6.2 | .92 | 15.5 | 13.1 | 13.1 | 13.3 | 13.7 | | | | |
| | | | | 6.0 | 5.0 | 5.4 | .93 | 15.2 | 13.1 | 12.4 | 12.1 | 13.2 | | | | |
| ¾ $HCO_3$ [8] | | | | 6.2 | 4.7 | 4.8 | .98 | | | | | | | | | |
| | | | | 5.6 | 5.2 | 6.1 | .85 | | | | | | | | | |
| 9966 | Aug. 14 | 38.0 | 10.0 | 9.9 | 4.8 | 5.5 | .87 | 19.6 | 16.2 | 14.6 | 13.2 | 15.9 | | | | |
| | | | | 9.8 | 5.2 | 5.7 | .91 | 17.9 | 16.3 | 15.3 | 12.6 | 15.5 | | | | |
| | | | | 8.9 | 4.3 | 4.6 | .94 | 16.7 | 15.9 | 13.7 | 12.8 | 14.8 | | | | |
| 9968 | Aug. 21 | 38.0 | 11.0 | 3.4 | 5.9 | 6.3 | .94 | 19.5 | 15.1 | 13.6 | 13.9 | 15.5 | | | | |
| | | | | 3.2 | 5.7 | 6.3 | .90 | 16.7 | 14.2 | 12.5 | 13.8 | 14.3 | | | | |
| 10046 | Aug. 23 | 38.0 | 10.0 | 8.2 | 4.4 | 5.6 | .79 | 14.2 | 10.1 | 12.2 | 9.9 | 11.6 | | | | |
| | | | | 7.6 | 4.2 | 5.5 | .76 | 12.4 | 10.8 | 10.5 | 9.8 | 10.9 | | | | |
| | | | | 7.5 | 5.2 | 6.2 | .84 | | | | | | | | | |
| 10045 | Aug. 26 | 38.0 | 9.8 | 10.4 | 3.3 | 4.1 | .80 | 21.3 | 18.9 | 18.3 | 16.9 | 18.8 | | | | |
| | | | | 10.3 | 3.5 | 4.3 | .81 | 19.4 | 17.9 | 16.5 | 16.5 | 17.6 | | | | |
| | | | | 9.7 | 4.3 | 4.8 | .90 | 16.6 | 16.1 | 14.9 | 16.5 | 16.0 | | | | |

See footnotes at end of table.

TABLE 1.—*Standard metabolic determinations of chicken tumors*—Continued

CHICKEN TUMOR 11—Continued

| Chicken No. | Date | Temperature | Dry weight | Determinations: Manometric: Aerobic $Q^{O_2}_A$ | Aerobic $Q_{CO_2}$ | Aerobic $Q_{O_2}$ | Aerobic R. Q. | Anaerobic $Q^{N_2}_A$ Hour 1st | Anaerobic $Q^{N_2}_A$ Hour 2d | Anaerobic $Q^{N_2}_A$ Hour 3d | Anaerobic $Q^{N_2}_A$ Hour 4th | Anaerobic $Q^{N_2}_A$ Average | Chemical $Q^{N_2}_{LA}$ | Chemical $Q^{O_2}_{LA}$ | Chemical/Manometric $Q^{N_2}_{LA}/Q^{N_2}_A$ | Chemical/Manometric $Q^{O_2}_{LA}/Q^{O_2}_A$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *1940* | ° *C* | *Pct.* | | | | | | | | | | | | | |
| 10041 | Aug. 28 | 38.0 | 9.0 | 8.6 | 4.0 | 5.0 | .80 | 22.2 | 25.0 | 18.5 | 17.8 | 20.9 | | | | |
| | | | | 8.2 | 4.3 | 4.9 | .88 | 22.3 | 20.3 | 16.9 | 16.6 | 19.0 | | | | |
| Grand averages | | 38.0 | 10.0 | 7.3 | 4.8 | 5.5 | .88 | 17.6 | 15.5 | 14.4 | 13.6 | 15.3 | 14.9 | 5.9 | 1.04 | .88 |
| Determinations number | | | | 33 | 33 | 33 | 33 | 26 | 26 | 26 | 26 | 26 | 8 | 10 | 7 | 10 |
| Tumors number | | | | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 3 | 3 | 3 | 3 |
| **CHICKEN TUMOR 12** | | | | | | | | | | | | | | | | |
| 9662 | May 20 | 37.7 | 10.0 | 7.5 | 0.8 | 2.3 | 0.35 | 8.3 | 10.2 | 7.0 | 7.4 | 8.2 | 10.0 | 6.3 | 1.22 | 0.84 |
| | | | | 6.1 | .7 | 1.7 | .41 | 4.2 | 5.3 | 4.2 | 4.9 | 4.6 | 6.5 | 4.6 | 1.41 | .75 |
| Liver metastasis[9] | | | | [2] 1.9 | [2] 2.2 | [2] 2.5 | [2] .88 | [2] 3.9 | [2] 3.7 | [2] 2.3 | [2] 2.1 | [2] 3.0 | [2] 2.5 | | [2] .83 | |
| 9659 | May 29 | 37.5 | 13.0 | 7.5 | 1.5 | 1.9 | .79 | 13.7 | 9.4 | 8.7 | [1] 8.4 | 10.0 | | | | |
| | | | | 7.1 | 1.1 | 1.8 | .61 | 7.9 | 7.0 | 7.9 | [1] 7.7 | 7.6 | | | | |
| | | | | 5.4 | 1.5 | 1.7 | .88 | | | | | | | | | |
| 9747 | June 21 | 38.1 | 11.0 | 6.8 | | 2.0 | | 11.3 | 8.3 | 5.9 | 7.1 | 8.1 | | | | |
| | | | | 6.6 | 1.1 | 2.4 | .46 | 8.4 | 9.1 | 6.9 | 7.6 | 8.0 | | | | |
| | | | | 6.5 | .7 | 2.1 | .33 | 7.6 | 7.2 | 6.1 | 6.1 | 6.7 | | | | |
| | | | | 6.0 | | 1.7 | | | | | | | | | | |
| 9943 | July 26 | 38.3 | 10.8 | 10.4 | 3.9 | 7.2 | .54 | 15.6 | 16.5 | 11.2 | 11.1 | 13.6 | 16.0 | 11.1 | 1.18 | 1.07 |
| | | | | 5.6 | 5.1 | 5.0 | 1.02 | 16.7 | 13.9 | 10.5 | 10.2 | 12.8 | 15.3 | 6.6 | 1.18 | 1.18 |
| | | | | 4.0 | 5.6 | 5.2 | 1.08 | 10.3 | 11.2 | 9.5 | 9.5 | 10.1 | 11.5 | 3.4 | 1.14 | .85 |
| 9947 | Aug. 12 | 37.9 | 10.0 | 7.2 | 3.3 | 4.6 | .72 | 8.4 | 7.7 | 5.3 | 6.6 | 7.0 | | | | |
| | | | | 6.0 | 2.5 | 3.5 | .72 | 4.6 | 4.3 | 3.7 | 3.1 | 3.9 | | | | |
| | | | | 4.5 | 2.9 | 3.5 | .83 | 4.9 | 6.5 | 4.2 | 5.2 | 5.2 | | | | |
| 10047 | Sept. 9 | 38.0 | 9.0 | 6.9 | 3.0 | 4.5 | .67 | 9.5 | 9.2 | 7.2 | 6.2 | 8.0 | 7.8 | 5.8 | .97 | .84 |
| | | | | 6.8 | 3.6 | 4.9 | .73 | 6.6 | 6.6 | 5.9 | 4.7 | 5.9 | 5.1 | | .86 | |
| | | | | | | | | 6.1 | 5.9 | 5.6 | 5.1 | 5.7 | 4.9 | | .86 | |
| 10049 | Sept. 27 | 38.0 | 10.0 | 5.3 | 1.9 | 2.8 | .68 | 7.3 | 5.3 | 5.3 | 5.3 | 5.9 | | | | |
| | | | | 4.3 | 1.8 | 2.4 | .75 | 5.6 | 5.0 | 5.3 | 5.0 | 5.2 | | | | |
| Grand averages | | 37.9 | 10.5 | 6.3 | 2.5 | 3.2 | .68 | 8.7 | 8.3 | 6.7 | 6.7 | 7.6 | 9.6 | 6.3 | 1.10 | .92 |
| Determinations number | | | | 19 | 17 | 19 | 17 | 18 | 18 | 18 | 18 | 18 | 8 | 6 | 8 | 6 |
| Tumors number | | | | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 3 | 3 | 3 | 3 |
| **CHICKEN TUMOR 13** | | | | | | | | | | | | | | | | |
| 9488 | Apr. 3 | 37.5 | 9.8 | 10.8 | 2.6 | 3.8 | 0.68 | 19.3 | 16.0 | 16.6 | 16.6 | 17.1 | 15.3 | 10.3 | 0.89 | 0.95 |
| | | | | 8.5 | 1.4 | 2.0 | .70 | 18.7 | 15.3 | 16.0 | 15.3 | 16.3 | 15.6 | 7.5 | .94 | .88 |
| | | | | | | | | 17.2 | 14.9 | 14.6 | 13.9 | 15.1 | 14.4 | | .95 | |
| | | | | | | | | 14.4 | 11.4 | 12.7 | 11.7 | 12.5 | 11.8 | | .94 | |
| 9507 | Apr. 15 | 39.0 | 8.6 | 10.6 | 3.4 | 2.8 | 1.21 | 19.9 | 16.4 | 15.4 | 14.7 | 16.6 | 19.2 | 9.6 | 1.16 | .90 |
| | | | | 9.0 | 3.5 | 3.2 | 1.09 | 17.9 | 16.1 | | | 17.0 | 20.1 | 7.4 | 1.18 | .82 |
| | | | | | | | | 10.5 | 11.8 | 9.4 | [1] 10.2 | 10.5 | 10.6 | | 1.01 | |
| No glucose | | | | [2] .86 | [2] 2.8 | [2] 3.9 | [2] .72 | [2] 2.9 | [2] .0 | [2] .0 | [2] .0 | [2] .7 | [2] .1 | [2]— .1 | [2] .14 | |
| | | | | [2] .64 | [2] 2.4 | [2] 3.1 | [2] .77 | [2] 2.2 | [2] .3 | | | [2] 1.2 | [2] .15 | [2]— .1 | [2] .12 | |
| | | | | | | | | [2] .9 | [2] .7 | [2] .7 | [2] 1.5 | [2] .9 | [2] .15 | | [2] .17 | |

See footnotes at end of table.

TABLE 1.—*Standard metabolic determinations of chicken tumors*—Continued

CHICKEN TUMOR 13—Continued

| Chicken No. | Date | Temperature | Dry weight | Determinations: Manometric: Aerobic: $Q^{O_2}{}_A$ | Aerobic: $Q_{CO_2}$ | Aerobic: $Q_{O_2}$ | Aerobic: R. Q. | Anaerobic: $Q^{N_2}{}_A$ Hour: 1st | 2d | 3d | 4th | Average | Chemical: $Q^{N_2}{}_{LA}$ | Chemical: $Q^{O_2}{}_{LA}$ | Chemical/Manometric: $Q^{N_2}{}_{LA}/Q^{N_2}{}_A$ | Chemical/Manometric: $Q^{O_2}{}_{LA}/Q^{O_2}{}_A$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *1940* | *° C.* | *Pet.* | | | | | | | | | | | | | |
| 9527 | Apr. 17 | 39.0 | 9.5 | 11.4 | | 3.9 | | [10] 8.9 | [10] 9.1 | [10] 7.6 | [10] 6.5 | [10] 8.0 | [2] 7.8 | | [2] .97 | |
| | | | | 8.9 | 2.1 | 2.7 | .78 | [10] 9.7 | [10] 8.0 | [10] 6.3 | [10] 6.3 | [10] 7.6 | [2] 7.4 | | [2] .97 | |
| | | | | 7.3 | 1.1 | 2.2 | .50 | [10] 5.1 | [10] 5.1 | [10] 3.5 | [10] 4.7 | [10] 4.6 | [2] 5.2 | | [2] 1.13 | |
| | | | | 6.9 | 3.4 | 2.9 | 1.17 | [10] 1.6 | [10] 4.1 | [10] 3.7 | [10] 5.4 | [10] 3.7 | [2] 4.4 | | [2] 1.19 | |
| | | | | | | | | [10] 1.8 | [10] 3.9 | [10] 3.9 | [10] 3.7 | [10] 3.3 | [2] 4.0 | | [2] 1.21 | |
| | | | | | | | | [10] 1.0 | [10] 1.0 | [10] 1.6 | [10] 1.3 | [10] 1.2 | [2] 1.4 | | [2] 1.17 | |
| | | | | | | | | [10] .7 | [10] .7 | [10] 1.0 | [10] 1.0 | [10] .8 | [2] .8 | | [2] 1.00 | |
| 9521 | Apr. 24 | 38.1 | 13.6 | 9.3 | 4.3 | 5.0 | .86 | 18.7 | 14.1 | 12.9 | [1] 10.1 | 13.9 | | | | |
| | | | | 6.1 | | 5.2 | | 16.0 | 13.1 | 10.8 | [1] 10.0 | 12.5 | | | | |
| | | | | 5.8 | 6.0 | 7.4 | .81 | | | | | | | | | |
| 9698 | May 4 | 37.5 | 10.4 | 10.4 | 2.7 | 4.2 | .64 | 13.2 | 16.2 | 14.8 | 12.4 | 14.1 | | | | |
| | | | | 9.3 | 2.4 | 3.9 | .62 | 14.5 | 15.1 | 13.6 | 12.4 | 13.9 | | | | |
| | | | | 7.0 | 3.1 | 3.1 | 1.00 | 13.2 | 13.4 | 12.3 | 11.2 | 12.5 | | | | |
| | | | | 6.9 | 3.6 | 3.3 | 1.09 | 12.8 | 11.3 | 11.6 | 10.6 | 11.6 | | | | |
| 9690 | May 15 | 38.2 | 7.9 | [5] 8.9 | [5] 1.9 | [5] 2.9 | [5] .66 | 19.1 | 18.9 | 16.8 | 14.1 | 17.2 | 16.1 | [5] 6.6 | .93 | .74 |
| | | | | 7.2 | 2.1 | 2.1 | 1.00 | [5] 18.1 | [5] 17.4 | [5] 15.4 | [5] 15.1 | 16.5 | [5] 16.2 | 6.2 | .98 | .86 |
| | | | | 5.9 | 2.4 | | | [5] 15.2 | [5] 16.5 | [5] 15.2 | [5] 15.7 | 15.6 | [5] 13.9 | 4.5 | .89 | .76 |
| 9702 | May 24 | 37.5 | 12.5 | 5.9 | 2.4 | 3.0 | .80 | 13.8 | 14.5 | 14.3 | 14.3 | 14.2 | 18.9 | 4.8 | 1.33 | .81 |
| | | | | 5.4 | 2.4 | 2.7 | .89 | 12.0 | 12.7 | 13.0 | 13.2 | 12.7 | | 5.0 | | .93 |
| | | | | | | | | 10.0 | 10.5 | 11.7 | 10.9 | 10.8 | 13.2 | | 1.22 | |
| 9856 | June 12 | 37.6 | 11.6 | 7.2 | 3.6 | 3.9 | .92 | 18.0 | 15.7 | 13.4 | 14.1 | 15.3 | | | | |
| | | | | 6.8 | 3.3 | 3.6 | .92 | 18.5 | 14.9 | 14.3 | 13.4 | 15.3 | | | | |
| | | | | 6.6 | 3.7 | 4.2 | .88 | 16.7 | 14.4 | 13.1 | 12.1 | 14.1 | | | | |
| | | | | 6.3 | 3.9 | 4.1 | .95 | | | | | | | | | |
| 9853 | June 15 | 38.0 | 7.9 | 11.4 | 4.6 | 5.3 | .87 | 26.1 | 21.6 | 20.0 | 12.6 | 20.1 | 19.1 | 8.8 | .95 | .77 |
| | | | | 9.6 | 3.2 | 3.9 | .82 | 24.3 | 19.1 | 19.9 | 12.6 | 19.0 | 21.4 | 7.2 | 1.13 | .75 |
| | | | | 8.8 | 5.1 | 5.4 | .94 | 23.3 | 19.1 | 17.7 | 12.5 | 18.1 | 15.9 | 7.1 | .88 | .81 |
| Grand averages | | 38.0 | 10.2 | 8.1 | 3.1 | 3.7 | .86 | 16.8 | 15.2 | 14.4 | 12.9 | 14.9 | 16.1 | 7.1 | 1.03 | .83 |
| Determinations | | | number | 27 | 25 | 26 | 24 | 25 | 25 | 24 | 24 | 25 | 15 | 12 | 15 | 12 |
| Tumors | | | number | 9 | 9 | 9 | 9 | 8 | 8 | 8 | 8 | 8 | 5 | 5 | 5 | 5 |

CHICKEN TUMOR I

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10288 | Oct. 9 | 38.0 | 8.0 | 9.4 | 1.0 | 1.8 | .56 | 13.9 | 13.5 | 13.1 | 13.1 | 13.4 | | | | |
| | | | | 8.6 | 1.1 | 1.7 | .65 | 13.1 | 13.1 | 12.2 | 12.2 | 12.6 | | | | |
| | | | | 8.6 | 1.1 | 1.7 | .65 | 10.3 | 10.7 | 11.1 | 11.5 | 10.9 | | | | |
| 10284 | Oct. 21 | 38.0 | 12.0 | 12.1 | 2.1 | 3.2 | .63 | 20.0 | 16.8 | 16.5 | 15.1 | 17.1 | | | | |
| | | | | 12.1 | 1.5 | 3.8 | | 17.1 | 15.5 | 15.5 | 15.5 | 15.9 | | | | |
| 10285 | Oct. 21 | 38.0 | 13.0 | 8.3 | 2.8 | 3.6 | .78 | 17.7 | 12.3 | 12.9 | 11.9 | 13.7 | | | | |
| | | | | 6.9 | 2.3 | 3.0 | .77 | 14.8 | 13.4 | 13.8 | 11.5 | 13.4 | | | | |
| 10287 | Oct. 21 | 38.0 | 12.0 | 12.1 | 2.2 | 3.7 | .60 | 27.4 | 23.6 | 22.6 | 21.3 | 23.7 | | | | |
| | | | | 10.4 | 1.7 | 2.1 | .78 | 23.9 | 23.2 | 22.2 | 20.9 | 22.5 | | | | |
| 2552 | Nov. 6 | 38.0 | 7.0 | 11.0 | 1.6 | 2.3 | .70 | 27.8 | 20.9 | 18.5 | 14.8 | 20.5 | 23.8 | 9.1 | 1.16 | .83 |
| | | | | 9.9 | 1.2 | 1.5 | .80 | 17.1 | 15.8 | 17.4 | 14.1 | 16.1 | 23.6 | 7.7 | 1.47 | .78 |
| 2553 | Nov. 6 | 38.0 | 8.0 | 11.0 | 1.7 | 2.8 | .61 | 17.5 | 14.8 | 14.8 | 11.8 | 14.7 | 17.1 | 11.9 | 1.16 | 1.48 |
| | | | | 9.4 | 1.3 | 2.1 | .62 | 17.2 | 16.4 | 14.5 | 10.0 | 14.5 | | 7.7 | | .82 |

See footnotes at end of table.

TABLE 1.—*Standard metabolic determinations of chicken tumors*—Continued

CHICKEN TUMOR I—Continued

| Chicken No. | Date | Temperature | Dry weight | Determinations: Manometric: Aerobic $Q^{O_2}_A$ | $Q_{CO_2}$ | $Q_{O_2}$ | R. Q. | Anaerobic $Q^{N_2}_A$: Hour 1st | 2d | 3d | 4th | Average | Chemical $Q^{N_2}_{I.A}$ | $Q^{O_2}_{I.A}$ | Chemical/Manometric $Q^{N_2}_{I.A}/Q^{N_2}_A$ | $Q^{O_2}_{I.A}/Q^{O_2}_A$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *1940* | *° C.* | *Pct.* | | | | | | | | | | | | | |
| 2549 | Nov. 8 | 38.0 | 6.7 | 10.9 | 1.6 | 2.9 | .55 | 20.9 | 15.1 | 14.1 | 14.1 | 16.0 | | | | |
| | | | | 10.0 | 1.1 | 2.5 | .44 | 17.3 | 13.9 | 10.4 | 10.4 | 13.0 | | | | |
| 2550 | Nov. 8 | 38.0 | 6.9 | 9.7 | 1.0 | 1.4 | .71 | 18.6 | 16.3 | 17.2 | 17.6 | 17.4 | | | | |
| | | | | 8.7 | 1.3 | 2.1 | .62 | 17.9 | 14.7 | 15.6 | 15.2 | 15.8 | | | | |
| 2551 | Nov. 8 | 38.0 | 12.0 | 10.8 | 2.8 | 3.2 | .88 | 20.3 | 17.3 | 17.4 | 15.3 | 17.6 | | | | |
| | | | | 9.7 | 2.2 | 2.7 | .81 | 13.0 | 11.2 | 11.4 | 10.4 | 11.5 | | | | |
| Grand averages | | 38.0 | 9.5 | 10.0 | 1.7 | 2.5 | .67 | 18.2 | 15.7 | 15.3 | 14.0 | 15.8 | 21.5 | 9.1 | 1.26 | .88 |
| Determinations number | | | | 19 | 19 | 19 | 18 | 19 | 19 | 19 | 19 | 19 | 3 | 4 | 3 | 4 |
| Tumors number | | | | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 2 | 2 | 2 | 2 |
| **CHICKEN TUMOR I CULTURED ON EGG EMBRYO** | | | | | | | | | | | | | | | | |
| O (6 eggs): | Nov. 13 | 38.0 | | | | | | | | | | | | | | |
| Nodular | | | 8.0 | 14.6 | 4.1 | 5.9 | .70 | 21.6 | 19.4 | 12.3 | 15.6 | 17.2 | | | | |
| | | | | 10.7 | 5.4 | 5.7 | .95 | 17.9 | 14.7 | 15.5 | 14.7 | 15.7 | | | | |
| Membranous | | | 8.0 | 5.3 | 4.8 | 2.8 | 1.71 | 11.6 | 11.1 | 10.4 | 10.7 | 10.9 | | | | |
| | | | | 4.4 | 5.1 | 3.6 | 1.42 | 9.7 | 9.5 | 9.2 | 10.0 | 9.6 | | | | |
| P (9 eggs): | Nov. 18 | 37.8 | | | | | | | | | | | | | | |
| Nodular | | | 6.4 | 8.6 | 2.3 | 3.2 | .72 | 18.0 | 17.3 | 17.3 | 14.7 | 16.8 | 18.7 | 5.8 | 1.11 | .67 |
| | | | | 6.8 | 5.6 | 4.1 | 1.37 | 12.9 | 12.3 | 14.3 | 10.9 | 12.6 | 13.7 | 5.3 | 1.09 | .78 |
| | | | | 6.1 | 7.1 | 5.8 | 1.23 | | | | | | | 7.1 | | 1.16 |
| NaCN [1] | | | | | | | | [2] 14.4 | [2] 13.8 | [2] 15.9 | [2] 11.2 | [2] 13.8 | [2] 16.9 | | [2] 1.22 | |
| "Hemorrhagic" | | | 6.5 | 6.8 | 3.6 | 3.0 | 1.20 | 17.3 | 20.5 | 16.5 | 17.8 | 18.0 | 22.8 | 6.4 | 1.26 | .94 |
| | | | | 4.9 | 5.8 | 3.6 | 1.61 | 11.1 | 9.8 | 15.6 | 9.8 | 11.6 | 10.9 | 3.5 | .94 | .71 |
| Membranous | | | 8.7 | 2.8 | 6.9 | 5.6 | 1.23 | 6.2 | 5.8 | 8.6 | 6.7 | 6.8 | | 2.6 | | .93 |
| | | | | | | | | 2.8 | 3.8 | 7.7 | 5.9 | 5.0 | 6.7 | | 1.34 | |
| Q (8 eggs): | Nov. 23 | 38.0 | | | | | | | | | | | | | | |
| Nodular | | | 7.0 | 9.9 | 4.7 | 4.9 | .96 | 22.2 | 19.5 | 17.6 | 16.9 | 19.0 | | | | |
| | | | | 9.6 | 4.5 | 4.9 | .92 | 18.5 | 16.6 | 15.1 | 15.5 | 16.4 | | | | |
| | | | | 9.0 | 4.0 | 4.2 | .95 | | | | | | | | | |
| Semimembranous | | | 7.5 | 6.0 | 2.6 | 3.2 | .81 | 18.1 | 17.7 | 15.1 | 14.4 | 16.3 | | | | |
| | | | | 5.5 | 2.9 | 3.5 | .83 | 13.5 | 12.9 | 11.4 | 12.1 | 12.5 | | | | |
| R (12 eggs): | Nov. 28 | 38.0 | | | | | | | | | | | | | | |
| Nodular | | | 6.7 | 12.9 | 3.4 | 5.6 | .61 | | | | | | | 12.4 | | .96 |
| | | | | 7.9 | 4.5 | 4.4 | 1.02 | | | | | | | 4.2 | | .53 |
| | | | | 7.4 | 4.2 | 4.4 | .96 | | | | | | | 7.6 | | 1.03 |
| "Hemorrhagic" | | | 7.4 | 12.6 | 2.2 | 4.3 | .51 | | | | | | | 11.7 | | .93 |
| | | | | 7.2 | 3.0 | 3.3 | .91 | | | | | | | 6.2 | | .86 |
| Membranous | | | 6.5 | 6.9 | 3.9 | 4.9 | .80 | | | | | | | 8.0 | | 1.16 |
| | | | | 4.5 | 3.0 | 4.1 | .73 | | | | | | | 5.4 | | 1.20 |

See footnotes at end of table.

TABLE 1.—*Standard metabolic determinations of chicken tumors*—Continued

CHICKEN TUMOR I CULTURED ON EGG EMBRYO—Continued

| Chicken No. | Date | Temperature | Dry weight | Determinations | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Manometric | | | | | | | | | Chemical | | Chemical/Manometric | |
| | | | | Aerobic | | | | Anaerobic | | | | | | | | |
| | | | | | | | | $Q^{N_2}{}_A$ | | | | | | | | |
| | | | | | | | | Hour | | | | | | | | |
| | | | | $Q^{O_2}{}_A$ | $Q_{CO_2}$ | $Q_{O_2}$ | R. Q. | 1st | 2d | 3d | 4th | Average | $Q^{N_2}{}_{LA}$ | $Q^{O_2}{}_{LA}$ | $Q^{N_2}{}_{LA}/Q^{N_2}{}_A$ | $Q^{O_2}{}_{LA}/Q^{O_2}{}_A$ |
| Nodular: | | | | | | | | | | | | | | | | |
| Grand averages | *1940* | ° *C.* | *Pct.* | | | | | | | | | | | | | |
| | | 38.0 | 7.0 | 9.4 | 4.5 | 4.9 | .94 | 18.5 | 16.6 | 15.3 | 14.7 | 16.3 | 16.2 | 7.1 | 1.10 | .86 |
| Determinations number | | | | 11 | 11 | 11 | 11 | 6 | 6 | 6 | 6 | 6 | 2 | 6 | 2 | 6 |
| Materials [12] number | | | | 4 | 4 | 4 | 4 | 3 | 3 | 3 | 3 | 3 | 1 | 2 | 1 | 1 |
| "Hemorrhagic": | | | | | | | | | | | | | | | | |
| Grand averages | | 38.0 | 6.9 | 7.9 | 3.6 | 3.5 | 1.06 | 14.2 | 15.1 | 16.0 | 13.8 | 14.8 | 16.8 | 6.9 | 1.10 | .86 |
| Determinations number | | | | 4 | 4 | 4 | 4 | 2 | 2 | 2 | 2 | 2 | 2 | 4 | 2 | 4 |
| Materials [12] number | | | | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 2 |
| Semimembranous: | | | | | | | | | | | | | | | | |
| Grand averages | | 38.0 | 7.5 | 5.7 | 2.7 | 3.3 | .82 | 15.8 | 15.3 | 13.2 | 13.2 | 14.4 | | | | |
| Determinations number | | | | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | | | | |
| Materials [12] number | | | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | | |
| Membranous: | | | | | | | | | | | | | | | | |
| Grand averages | | 38.0 | 7.7 | 4.8 | 4.7 | 4.2 | 1.18 | 7.6 | 7.5 | 9.0 | 8.3 | 8.1 | 6.7 | 5.3 | 1.34 | 1.10 |
| Determinations number | | | | 5 | 5 | 5 | 5 | 4 | 4 | 4 | 4 | 4 | 1 | 3 | 1 | 3 |
| Materials [12] number | | | | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 1 | 2 |

[1] Readings taken for fifth, sixth, and even eighth hour, with relatively little further decrease.

[2] Not included in grand averages.

[3] Portion of tumor material irradiated, immediately after removal from chicken, with 20,000 r units over period of about 30 minutes.

[4] Material allowed to stand at 10° C. for 24 hours before manometric analysis (material of Oct. 14, 1940).

[5] Unrinsed, i. e., tissue slices placed directly into experimental vessels without usual preliminary standing in bicarbonate-glucose-free Ringer's solution.

[6] Slices placed in Ringer-bicarbonate-glucose medium in vessels on manometers and maintained in 95 percent $N_2$-5 percent $CO_2$ for 1 hour and then later replaced by 95 percent $O_2$-5 percent $CO_2$ and the experiment conducted as usual.

[7] As in footnote 6 but with glucose withheld during the 1-hour anaerobiosis.

[8] Bicarbonate concentration three-fourths usual value.

[9] Subsequent histological examination of aliquots of the material metabolized indicated that somewhat more than half the sliced material consisted of liver, the rest being an invading metastasis of tumor 12. The metabolism values obtained are nearer those of liver than of tumor 12, evidently because of the actual proportions of the two tissues involved.

[10] Anaerobic values not included in grand averages. Glucose added to first three after preliminary ¾-hour anaerobiosis at $t=0$; to fourth and fifth after 1¼ hours at $t=\frac{1}{2}$; to sixth after 2¾ hours at $t=2$; and to seventh after 4½ hours at $t=3\frac{1}{2}$ hours. Respective values for fifth and sixth hours are 6.5 and 6.5; 6.3 and 5.9; 4.7 and 4.9; 5.4 and 5.4; 3.7 and 3.3; 1.3 and 0.7; 0 and 1.1. The average $QN_2{}_A$ includes these latter values, in order to correspond to the $QN_2{}_{LA}$ values reported.

[11] 0.0036 M NaCN; gas phase 5 percent $CO_2$ in 95 percent $O_2$ instead of in 95 percent $N_2$.

[12] A given material may contain several embryo tumors combined, where similar in gross appearance and individually insufficient for a complete series of determinations.

requiring little or no statistical treatment for their detection are being looked for, the limits of replicate variation and error may well call for somewhat wider tolerance than in many other more specialized studies, and indeed a relaxing of adherence to minor aspects of the standardized routine has desirable features. In this light, the replication obtained with the various Q values is, by and large, quite satisfactory, such differences as do exist probably being more interesting than undesirable, regardless of whether these differences may or may not be completely understood. The experimental data reported in table 1 are not selected but are presented essentially in entirety, in order to leave no doubt as to the extreme limits of experimental variation obtained.

In regard to the variability of replicates among the different types of aerobic Q values, it is our general impression that the variability increases in the order $Q_{O_2}$, $Q^{O_2}{}_A$, $Q_{CO_2}$, and R. Q., which is the order of difficulty of experimental determination. With the Summerson manometer, $Q_{CO_2}$ depends very markedly on the accuracy of the $h_1$ reading, and the R. Q. is similarly affected, but of course contains in addition any error and variability also introduced by the $Q_{O_2}$ values. The $Q_{O_2}$ values depend only on the $h_2$ readings, whereas the $Q^{O_2}{}_A$ and $Q_{CO_2}$ values each depend upon two readings, $h$ and $h_1$, and $h_1$ and $h_2$, respectively.

A considerable part of the variability between replicates is unquestionably due to unavoidable differences in the wet weight/dry weight ratios, caused by the impossibility of accomplishing strictly uniform blotting of the slices immediately before their weighing. Evidence confirming this consideration may be obtained from the fact that the arrangement of aerobic data in decreasing order of $Q^{O_2}{}_A$ values of replicates shows a surprising degree of correlation with the replicate $Q_{CO_2}$ and $Q_{O_2}$ values, which likewise fall in decreasing order, in at least 75 percent of the cases. It scarcely seems likely that this high correlation could be obtained except on some basis, such as percent of moisture, that would automatically affect all of the calculated Q values in the same direction. An inverse correlation of order of $Q^{O_2}{}_A$ with $Q_{CO_2}$ and $Q_{O_2}$ values could be expected if the $Q^{O_2}{}_A$ values were varying mainly with the percentage purity of tumor in the slices. Thus, a high percentage of tumor cells in a given replicate slice of material yielding a higher $Q^{O_2}{}_A$ value could be expected to yield relatively lower $Q_{CO_2}$ and $Q_{O_2}$ values than in another replicate sample with fewer tumor cells, since tumor metabolism is in general characterized by relatively high $Q^{O_2}{}_A$ values, and low $Q_{O_2}$ and particularly low $Q_{CO_2}$ values (low R. Q. values).[5]

Another factor concerned in the aerobic determination is that, with tumors possessing relatively low $Q_{O_2}$ or $Q_{CO_2}$ values, or relatively high $Q^{O_2}{}_A$ values, it becomes more difficult to adjust the amount of tissue employed so that it will be optimum for determining all three quantities. To

[5] *In the case of tumor 13, it is possible that a negative correlation actually does exist between given replicate values of $Q^{O_2}{}_A$ and $Q_{CO_2}$ (likewise $Q^{O_2}{}_A$ and R. Q.), a phenomenon that we seem to have observed to some extent also in rat liver cirrhoses and carcinomas induced by dimethylaminoazobenzene. If this phenomenon is genuine, it is probably not the methodological one that might be caused simply by faulty $h_1$ readings, since this would change $Q^{O_2}{}_A$ and $Q_{CO_2}$ by equal and opposite amounts, whereas the increase in $Q_{CO_2}$ is usually several fold greater than the decrease in $Q^{O_2}{}_A$. This suggests that some intermediate acid product (possibly a dicarboxylic acid) containing several carbon atoms is, in the replicate with the lower $Q^{O_2}{}_A$ value, being converted into carbon dioxide: in other words, for some reason more complete oxidation or decarboxylation of the intermediate has occurred. Whether oxidation or decarboxylation, or both, is involved in any given instance, might be decided by a consideration of the replicate $Q_{O_2}$ values. The data available do not permit more than an indefinite decision at present. It is important to note that if the phenomenon described is metabolically real, it would also account for part of the variability in R. Q.*

use enough tissue to obtain satisfactorily large values of total oxygen consumption and carbon dioxide production ($X_{O_2}$ and $X_{CO_2}$ values) would yield at the same time relatively large $X^{O_2}{}_A$ values with correspondingly large decreases in pH, which is not desirable. Much of the large variation in the R. Q. values of tumors 12 and 13, especially in the first experiments, is unquestionably due to the fact that the amounts of tissue employed were adjusted to yield appropriate $X^{O_2}{}_A$ values, but too small $X_{O_2}$ and $X_{CO_2}$ values, and hence highly variable and relatively inaccurate $Q_{O_2}$, $Q_{CO_2}$, and R. Q. values.

*Anaerobic Q Values*

Except for composition differences in the slices, anaerobic replication should be very close; and if in the table an exceptionally low replicate value is found, it may be regarded with the suspicion that it is a result of imperfect removal of all oxygen during equilibration. The presence of 1 percent or less residual oxygen in a vessel would scarcely reduce the actual rate of acid production, but the consumption of this oxygen by the tissue would result in a pressure change of reversed sign, and would thus yield a $Q^{N_2}{}_A$ value lower than the correct one. For this reason, the higher replicate values are probably to be preferred.

The anaerobic $Q^{N_2}{}_A$ values are given hourly for some 4 hours in order to indicate their rates of fall with time, which are, in fact, as with most tumors, considerably less than with most adult normal tissues, especially liver. As can be seen, the tumors possess a fairly stable $Q^{N_2}{}_A$ rate over a period of many hours, decreasing seldom by more than 30 percent in 4 hours, sometimes not at all, and perhaps 10 to 15 percent as an average, as compared with 50 to 90 percent with normal liver, which of course possesses a much lower absolute $Q^{N_2}{}_A$ (and $Q_{LA}$ essentially equal to O). Some of this fall is doubtless methodological, involving decreasing glucose concentration, bicarbonate concentration, or pH; and as our investigations progressed, we found it preferable, for the amounts of tissue and time employed, to use at least 3 cc. of medium in the anaerobic vessels and 2 cc. in the aerobic, and even more if feasible.[6] We believe that the 4-hour average $Q^{N_2}{}_A$ value is probably as representative or more so than the value for the first hour or any other hour. In general it most nearly equals the second-hour value. The first-hour value may be "high" because of equilibration factors either inherent in the tissue or methodological (manometric). The average 4-hour (or indeed second-hour) $Q^{N_2}{}_A$ values are essentially equivalent to the 30-to-120-minute $Q^{N_2}{}_A$ values favored by some workers. Readings were not infrequently taken for periods longer than 4 hours (e. g., table 1, chicken tumors 16, 12, and 13) and the further decreases in $Q^{N_2}{}_A$ values observed were ordinarily even less than in the 2-to-4-hour period.

The old method of obtaining $Q^{N_2}{}_A$ in the presence of oxygen by the use of cyanide to inhibit respiration, introduced by Warburg (*2*) and employed in the studies of Murphy and Hawkins (*16*) and many others of that period, receives confirmation in table 1 (tumor 1 on egg embryo). For tumor P, the values of $Q^{N_2}{}_A$ with cyanide and oxygen are indistinguishable from those obtained anaerobically and without cyanide.

*Chemical Q Values*

The chemical lactic acid determinations serve to establish not only the absolute $Q_{LA}$ values but also to confirm the manometric

[6] *In table 1, tumor 11, No. 9967, the use of three-fourths of the customary initial bicarbonate concentration introduced no measurable change in any of the aerobically determined values.*

$Q^{N_2}{}_A$ values and differences between manometric replicates. Corresponding $Q^{N_2}{}_{LA}$ and $Q^{N_2}{}_A$ determinations were always performed on the same particular sliced material and for an identical period of time. Whereas the $Q^{N_2}{}_{LA}$ values refer, so far as is known, solely to lactic acid, as determined by the highly specific method of Barker and Summerson (27), the $Q^{N_2}{}_A$ values refer to net acid production (including base consumption, if any). With the individual tumor samples examined, the $Q_{LA}/Q_A$ values vary a good deal, and it is probable that all proper aerobic values lie between 0.7 and 1.0, and anaerobic values between 0.8 and 1.2, as extreme limits. Values outside these ranges must be regarded as unlikely; although, to be sure, with a tissue such as liver, values as low as zero may be real. The grand averages given in the individual tumor tables are calculated from the vertical chemical/manometric $Q_{LA}/Q_A$ columns and not from the horizontal grand average values, since on the latter basis unequal, noncorresponding numbers of $Q_{LA}/Q_A$ determinations would be concerned.

## Miscellaneous Variables

In table 1 will be found incidental experiments on items of special interest, including effects of high temperature, cyanide, bicarbonate and glucose concentrations, dry-weight procedure, etc. These incidental results are not included in the grand averages and are probably not specific for the particular tumor that happened to be employed.

### *Temperature*

It is probable that the incidental differences in temperature settings from day to day, ranging between 37° and 39° C., caused little variation between one sample of tumor transplant and another, as distinguished from replicates of a given sample, since this temperature range so closely approximates the optimum. As indicated in table 1, tumor 16 No. 9595, even 41° scarcely changed the glycolysis values as compared with those for 37°, although the respiration values were in this instance apparently increased. The respiratory quotient was not significantly affected, as noted earlier by Dickens and Simer (7) (cf. table 4, footnote). Forty-one degrees is, of course, closer to the chicken body temperature than the average 38° usually employed; but the latter temperature was chosen as being nearer temperatures previously used in studying mammalian tissue slices and was also adopted in view of several exploratory experiments at 41° which had indicated little difference between values there and at 37°. More work could undoubtedly be carried out concerning this matter.

### *Dry Weight*

The possible errors in percentage dry weight of each tumor sample probably introduce considerably more variability between tumors than do the temperature differences just discussed, in the results obtained with different transplants of a given tumor. The percent dry-weight values must be regarded, however, as fairly constant for any given tumor, and the grand average values were indeed nearly the same for all tumors, namely, about 10 percent, except in the case of the methylcholanthrene-induced tumor 16, which was 40 percent greater, and the embryo-cultured tumor 1, which was 30 percent less.

In all our experiments, the initial dry weight has been taken as the basis of calculation for the metabolic values, although until a few years ago the final dry weight was almost invariably employed by others, following the original procedure introduced by Warburg. The final dry

weight is usually much smaller than the initial dry weight [7] and therefore gives much larger Q values. Whether to use the initial dry weight or the final dry weight (obtained by calculation of dry weight of residual slices removed from vessels at the end of an experiment) as a basis of establishing the metabolic values, has been a point of some discussion in the past. Elliott, Greig, and Benoy (*37*, *38*) discuss the advantages and disadvantages of both methods. In our opinion, use of the final dry weight is scarcely ever to be recommended and ordinarily to be deplored. The main argument advanced in its favor, that the residual intact tissue is essentially responsible for the metabolism, and the diffuse material not appreciably, is highly gratuitous, and in any event the final dry-weight values refer only to the residual intact tissue at the end, and not during the average course of the experiment. Furthermore, even in a single set of experiments, the wet weight/dry weight ratio for a sample of tissue in different manometer vessels varies widely (*37*, *38*, *39*). Technical difficulties in recovering the tissue from the medium also reduce accuracy, especially where relatively small amounts of tissue slice material for dry-weight weighings are involved, which is frequently the case. The initial dry weight is unquestionably more representative of the fresh tissue and is obtained with far less trouble.

Table 2 shows figures for both initial and final dry weight determinations on aliquots of the same original tissue, together with the desired final/initial dry weight ratio. The average final/initial ratio is about 0.5, which represents a very marked "correction factor" and one which in principle introduces a great deal of uncertainty into many, possibly the bulk, of the Q values reported in the literature during the past 20 years. The situation is undoubtedly further aggravated by the fact that the correction must show considerable numerical variation from tissue to tissue, and from the experimental conditions of one investigator to another, and in particular with the length of time and vigorousness of shaking involved. In comparing our Q values, or those of others likewise based upon initial dry weights, with any other reported Q values based upon final dry weights, we do not hesitate to recommend multiplication of the final dry-weight value by a factor definitely less than unity, possibly 0.8 for the least fragile tissues, and even as low as 0.3 for the most fragile tissues (cf. *39*.) For experiments with tumors involving at least 1 hour's shaking, a factor of from 0.5 to 0.6 would be appropriate; and as indicated in table 2, we have applied this correction to all chicken tumor metabolism data previously reported, all of which appear to be based upon final dry weights, that is, the "procedure of Warburg" was reported to have been followed.

[7] *With some tissues the final wet weight is sometimes actually larger than the initial wet weight. This may occur when the dry weight loss is relatively small, and is caused by the taking up of fluid by the tissue during the experimental period.*

### *Rinsing*

As indicated in table 1, tumor 11, No. 9948 and tumor 13, No. 9690, the effect of the customary rinsing or standing, during the process of slicing, at room temperature or lower in bicarbonate-phosphate-glucose-free Ringer's solution, as compared with placing the tissue slices directly into the vessels, had no marked effect on the glycolysis values. The R. Q. values, however, tended to become higher with prolonged rinsing, although the increase was quite out of the ordinary in the particular instances given. The rise was caused mainly by the increase in $Q_{CO_2}$ values and also possibly by the fact that

TABLE 2.—*Ratio of final to initial dry weight (chicken tumor 1* [1])

| Tumor | Initial weight [2] | | | Final weight [3] | | | Final/initial weight [4] |
|---|---|---|---|---|---|---|---|
| | Wet | Dry | Dry | Wet | Dry | Dry | Dry |
| | *Mg.* | *Mg.* | *Pct.* | *Mg.* | *Mg.* | *Pct.* | *Percent* |
| Chick-cultured: | | | | | | | |
| No. 10284 | 100 | 12.0 | 12.0 | [6] 101 | 6.0 | 5.9 | 49 |
| No. 2553 | 100 | 8.0 | 8.0 | 101 | 3.9 | 3.9 | 49 |
| Embryo-cultured: | | | | | | | |
| O: | | | | | | | |
| Nodular | 100 | 8.0 | 8.0 | 105 | 5.8 | 5.5 | 69 |
| | | | | 102 | 5.5 | 5.4 | 68 |
| P: | | | | | | | |
| Nodular | 200 | 12.8 | 6.4 | [5] 258 | 8.3 | 3.2 | 50 |
| "Hemorrhagic" | 200 | 13.0 | 6.5 | [5] 253 | 8.7 | 3.4 | 52 |
| Membranous | 65 | 5.7 | 8.8 | [5] 253 | 8.8 | 3.5 | 40 |
| Q: | | | | | | | |
| Nodular | 200 | 14.0 | 7.0 | [5] 300 | 10.0 | 3.3 | 47 |
| | | | | [6] 51 | 1.7 | 3.3 | 47 |
| | | | | [6] 50 | 1.8 | 3.6 | 51 |
| R: | | | | | | | |
| Nodular | 203 | 13.5 | 6.7 | [5] 603 | 22.0 | 3.6 | 54 |
| "Hemorrhagic" | 203 | 15.0 | 7.4 | [5] 607 | 23.0 | 3.8 | 51 |
| Membranous | 200 | 13.0 | 6.5 | [5] 604 | 24.0 | 4.0 | 61 |

[1] Similar values have been obtained with butter-yellow-induced rat-liver cirrhoses, tumors, and necroses, and normal rat liver; and normal and leukemic mouse liver, spleen, and lymph node.
[2] Taken on aliquot portions of materials used in the manometers.
[3] Wet weight is of actual sample put into vessel at beginning of manometric run; dry weight is of sample after removing at end of run, drying and weighing.
[4] Wet weights of duplicates at end of run (before drying) 65 and 68 mg., respectively, or losses of 39 and 43 mg. (38 and 43 percent).
[5] Tissues from 2 or 4 vessels combined.
[6] Anaerobic vessels; all others aerobic.

the $Q_{O_2}$ values were slightly lowered. In view of this effect of rinsing upon R. Q., the tumor R. Q. values reported in the table may be regarded as probably somewhat higher than those that would have been obtained if the tissue slices had been placed on moist filter paper in thermostated chambers during slicing. It is worth noting that with a tissue such as liver the effect of rinsing is contrary to that with tumors, the R. Q. being lowered with liver. Data concerning this will be presented and discussed in a forthcoming paper on metabolism of livers and hepatomas of rats fed *p*-dimethylaminoazobenzene (butter yellow). On the other hand, Shorr (*40*) observed that the R. Q. of excised cardiac tissue of diabetic dogs increases up to (but not higher than) unity, upon prolonged incubation in Ringer-phosphate solution (more rapidly in Ringer-glucose-phosphate solution, and at higher temperatures); the phosphate is essential for the rise to take place. As compared with liver, there would thus appear to be some resemblance, regarding increased R. Q. upon incubation, between tumor and excised diabetic tissue, both presumably being without hormonal control and with their respiratory activity ordinarily responding but little to the addition of carbohydrate. Much more work needs to be done on the effect of phosphate and other conditions on tumor R. Q. changes during incubation.

### *Absence of Glucose*

The effect of omission of glucose on Q values is illustrated for tumor 13 (table 1, tumor 13, No. 9507). The glycolysis, aerobic and anaerobic, is negligible, at least in terms of chemically determined lactic acid, whereas the oxygen consumption is slightly increased, and carbon dioxide and R. Q. are noticeably decreased. The effect of added glucose in decreasing $Q_{O_2}$ slightly has been observed by many workers with various tumors and normal tissues. In tumor 11 (table 3, No.10142) the effect is very considerable (50 percent), so marked that here $Q_{CO_2}$ is also reduced.

### *Preliminary Anaerobiosis*

Treatment of the tumor slices at body temperature under anaerobic conditions in the absence of glucose for varying periods of time above 1 hour produces a marked, progressively irreversible loss of ability to glycolyze anaerobically, as indicated in table 1, tumor 13, No. 9527. The inhibiting effect on aerobic glycolysis following such treatment is probably less marked (table 1, tumor 11, No. 9948). There is some suggestion that the $Q_{O_2}$ is slightly raised and the R. Q. slightly low-

ered, especially if no glucose has been present during preliminary anaerobiosis; the glucose is apparently somewhat beneficial for $Q^{O_2}{}_A$ if present during treatment. From these results one might suppose that some sort of apparent inhibition of the Pasteur effect could be obtained by preliminary anaerobiosis, in the sense that the anaerobic glycolysis would be more greatly reduced than the aerobic. The result would be that upon admission of oxygen after treatment, the difference between aerobic and anaerobic glycolysis would be reduced or wiped out or even become negative. Effects of substrate deprivation and preliminary anaerobiosis have been considered earlier by Dickens and Greville (*13*).

*X-radiation*

X-radiation of ice-cold methylcholanthrene-induced tumor 16 (table 1, Nos. 10181, and 10271–10275) with 20,000 r units over a period of 30 minutes produced no effect on the standard metabolic Q values when these were determined immediately after irradiation, even though transplants of such X-rayed material were no longer successful. However, when the irradiated tumor material was allowed to stand for a day at about 6° to 10° C., a very great reduction was observed in the aerobic $Q_{CO_2}$, $Q_{O_2}$, and R. Q. values. These results are somewhat contrary to those of Crabtree (*11*), who reported that irradiation of tumor tissue at 0° to 10° with radium caused an immediate selective lowering of glycolysis whereas the respiration remained relatively unaffected; upon irradiation at body temperature, however, glycolysis was reduced, but respiration was not affected. The unsuccessful attempts at transplantation, even if performed immediately after irradiation, were taken to be a very delicate test of the absence of a filterable agent in this tumor.

Lactate Utilization

The experimental values of some three to six for the Meyerhof oxidation quotient in tumors signify that each equivalent of oxygen gas consumed is *accompanied by* (not necessarily causes) a decrease in glycolysis under aerobic as compared with anaerobic conditions of three to six oxidizable equivalents (1 to 2 molecules) of lactic acid (*lactic* acid to the extent that $Q_A = Q_{LA}$). In terms of the old, now largely discarded Meyerhof Cycle theory, the decreased lactic acid would represent lactic acid disappearing by resynthesis to higher molecular weight carbohydrate; in terms of the Lipmann explanation, it would represent lactic acid never actually formed; a third possibility is actual formation followed by synthesis to materials other than the higher carbohydrate. That neither resynthesis nor synthesis is markedly involved is evident from results with tumor 11 in table 3, which shows, following earlier work by Benoy and Elliott (*12*) with Philadelphia No. 1 sarcoma and Walker No. 256 carcinoma, that lactate is utilized very slowly aerobically (and not at all anaerobically). The $Q^{O_2}{}_A$ and $Q^{O_2}{}_{LA}$ values scarcely attain $-1.5$ as compared with the absolute Pasteur effect values of $[Q^{N_2}{}_A - Q^{O_2}{}_{LA}] = 9.2$ (or $[Q^{N_2}{}_{LA}] - [Q^{O_2}{}_{LA}] = 9.0$). Lactic acid (or total acid) formation and subsequent disappearance, therefore, does not seem to explain at all quantitatively the observed Meyerhof oxidation quotients, and it is necessary to regard some explanation such as that of Lipmann (cf. Burk (*5*, *6*)) as obtaining.

An interesting feature of table 3 is that the chemical/manometric ratios (lactic acid/total acid) in the presence of added lactate (and absence of glucose) are quite different from those in the cases heretofore where glucose has been present. Anaerobically the values are no longer

TABLE 3.—*Aerobic and anaerobic lactate [1] utilization or consumption by chicken tumor 11 in glucose-free Ringer-bicarbonate medium [2]*

| Chicken No. | Date | Temperature | Dry weight | Lactate added | Determinations | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Manometric | | | | | Chemical | | Chemical/Manometric | |
| | | | | | Aerobic | | | | Anaerobic | | | | |
| | | | | | $Q^{O_2}{}_A$ | $Q_{CO_2}$ | $Q_{O_2}$ | R. Q. | $Q^{N_2}{}_A$ | $Q^{N_2}{}_{LA}$ | $Q^{O_2}{}_{LA}$ | $Q^{N_2}{}_{LA}/Q^{N_2}{}_A$ | $Q^{O_2}{}_{LA}/Q^{O_2}{}_A$ |
| | *1940* | °C. | *Pct.* | *Pct.* | | | | | | | | | |
| 10145 | Sept. 24 | 38.0 | 10.0 | 0.00 | −0.7 | 5.5 | 6.8 | 0.81 | | | −0.9 | | 1.29 |
| | | | | .02 | −1.0 | | | | | | −1.3 | | 1.30 |
| | | | | .06 | −.8 | 4.3 | 5.2 | .83 | | | −1.5 | | 1.87 |
| | | | | .20 | −1.6 | 7.2 | 8.0 | .90 | | | | | |
| | | | | .00 | | | | | +1.7 | +0.00 | | +0.00 | |
| | | | | .20 | | | | | +1.9 | +.10 | | +.05 | |
| 10142 | Sept. 25 | 38.0 | 10.0 | .00 | −.7 | 6.5 | 8.1 | .80 | | | −.9 | | 1.28 |
| | | | | .02 | −1.0 | 6.9 | 8.4 | .82 | | | −.9 | | .90 |
| | | | | .06 | −1.1 | 6.8 | 8.0 | .85 | | | −1.8 | | 1.64 |
| | | | | .20 | −1.8 | 7.7 | 8.2 | .94 | | | | | |
| | | | | .00 | | | | | +1.7 | +.00 | | +.00 | |
| | | | | .20 | | | | | +1.9 | +.10 | | +.05 | |
| Glucose (0.24 percent) | | | | [3].00 | [3]+6.4 | [3]3.8 | [3]4.1 | [3].93 | | | | | |
| Grand averages | | | | | −1.1 | 6.4 | 7.5 | .85 | +1.8 | −.05 | −1.2 | +.02 | 1.38 |
| Determinations....no | | | | | 8 | 7 | 7 | 7 | 4 | 4 | 6 | 4 | 6 |
| Tumors....no | | | | | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |

[1] Added as neutralized sodium lactate.
[2] All experimental periods (anaerobic as well as aerobic) 2 hours.
[3] Not included in grand averages.

about unity but essentially zero: some small amount of total acid is produced but no lactic. Aerobically the ratios are now somewhat greater than unity, rather than somewhat less, since the negative $Q^{O_2}{}_A$ values are less negative because of the positive production of total acid (which is evidently not utilized, at least greatly); the negative $Q^{O_2}{}_{LA}$ values, however, are not reduced by similar considerations and hence are now algebraically larger than the $Q^{O_2}{}_A$ values. The same factor, namely, aerobic production of acid besides lactic (in the presence or absence of glucose or lactate or both), thus operates both to make the ratios in glucose less than unity and those in lactate greater. The experiments of table 3 could be amplified to extend to higher concentrations of lactate (where greater utilization might conceivably be obtained) and even to other tumors. Had a considerable lactate utilization by tumors been observed, it was intended to make a detailed study as to its fate in disappearance, but under the circumstances the matter has not been pursued for the present.

## COMPARATIVE ANALYSIS OF ALL CHICKEN TUMOR METABOLISM DATA

The chief interest in the individual tumor data eventually focuses upon the grand averages of the many individual determinations. Statistical treatment of the grand averages was not deemed warranted, for the present at least. It may be said, however, that replicate determinations on a given tumor sample would appear to merit about the same weight as

an equal number of single determinations on different samples of the same kind of tumor and are so weighted in the grand averages. These grand averages are brought together for ready comparison in table 4, in which certain derived quotients and functions long useful in characterizing tumor metabolism are calculated. In addition, in the last section of the table and in a footnote are reported essentially all other chicken tumor metabolism data available from the literature. In considering the data of table 4 in comparison with data for other tumors and times, reference should be made to the extended discussion, analysis, and tabular summary thereof by Burk (*5*, *6*). More recent shorter discussions, and also controversy concerning various aspects of the nature of malignant tumor metabolism, are given by Dickens (*43*), Boyland (*44*), Berenblum, Chain, and Heatley (*45*), Dickens and Weil-Malherbe (*39*, *46*), and Burk, Behrens, and Sugiura (*47*). In the controversial issues the present writers are most nearly in agreement with the views of Dickens, and are to a large extent opposed to those of Berenblum, as will be considered elsewhere (*48* et seq.).

The first point of interest in table 4 is the very large magnitude of the $Q^{N_2}{}_A$ and $Q^{O_2}{}_A$ values (also $Q^{N_2}{}_{LA}$ and $Q^{O_2}{}_{LA}$ values) of all of the tumors except perhaps in the two cases of the tumors containing very considerable amounts of inactive material, the osteochondrosarcoma 12, and the membranous embryo-cultured tumor 1 that contains so few tumor cells. The $Q^{N_2}{}_A$ values (based on initial dry weights) are essentially as large as those for any previous groups of malignant tumors studied. The tumor with the highest value of all is the hard, compact No. 10, which conceivably was derived (via tumor 9) from coal-tar injection. The other tumor, definitely derived on a chemically carcinogenic basis, methylcholanthrene-induced tumor 16, also the most compact of the tumors, would, on a wet-weight basis (i. e., present values multiplied by about 14.2/10), provide a close second in relation to glycolysis.

The respiratory quotient of all chick-cultured tumors is well below unity, in accordance with the view introduced by Dickens regarding tumor metabolism, and, as discussed earlier in the text, the values reported may be systematically somewhat high. The R. Q. range is approximately 0.65 to 0.90. The respiratory $O_2$ and $CO_2$ ($Q_{O_2}$ and $Q_{CO_2}$) are very definitely low. The lowest $Q_{O_2}$ was obtained with tumor 1, with which Warburg had obtained considerable support for his view that the respiration of tumors was low; it has the lowest R. Q. of any chicken tumor. Furthermore the values obtained in this investigation for chicken-grown tumor 1 are strikingly close to those reported earlier by Warburg and by Murphy and Hawkins, when these latter values are corrected to initial rather than final dry weights. The other tumor studied by Murphy and Hawkins, No. 9, likewise yielded values in the same range as the tumors studied in this investigation, and the same may be said for the miscellaneous values reported for tumor 1 of Rous and Mill-Hill fowl sarcoma 1 in footnote 3 of table 4.

The nodular embryo-cultured tumor 1 possesses a glycolytic metabolism identical with that of the chick-grown tumor 1, but the respiratory $Q_{O_2}$, $Q_{CO_2}$, and R. Q. values are without question greatly increased, $Q_{CO_2}$ and $Q_{O_2}$ by twofold to threefold, and R. Q. nearly attains unity. Whatever influence is operating here to accomplish this continues with greater force in the membranous tumor, where the R. Q. is certainly well above unity. This material, however, is not to be considered as a case of a tumor with R. Q. above

TABLE 4.—*Summary of all standard metabolism values[1] available for chicken tumors studied 1925–41*

| Chicken tumor No. | Dry weight | Determinations: Manometric: Aerobic acid formation ($Q^{O_2}_A$) | Manometric: Carbon dioxide production ($Q_{CO_2}$) | Manometric: Oxygen consumption ($Q_{O_2}$) | Manometric: Respiratory quotient (R. Q.) | Manometric: Anaerobic acid formation ($Q^{N_2}_A$) | Chemical: Anaerobic lactic acid formation ($Q^{N_2}_{LA}$) | Chemical: Aerobic lactic acid formation ($Q^{O_2}_{LA}$) | Chemical/manometric: Anaerobic lactic acid/acid ratio ($Q^{N_2}_{LA}/Q^{N_2}_A$) | Chemical/manometric: Aerobic lactic acid/acid ratio ($Q^{O_2}_{LA}/Q^{O_2}_A$) | Derived functions: Absolute Pasteur effect ($Q^{N_2}_A - Q^{O_2}_A$) | Derived functions: Percent Pasteur effect $\left(\frac{100(Q^{N_2}_A - Q^{O_2}_A}{Q^{N_2}_A}\right)$ | Derived functions: Meyerhof oxidation quotient $\left(\frac{Q^{N_2}_A - Q^{O_2}_A}{Q_{O_2}/3}\right)$ | Derived functions: Extent quotient $\left(\frac{Q^{N_2}_A}{Q_{O_2}/3}\right)$ | Derived functions: Fermentation excess ($U = Q^{N_2}_A - 2Q_{O_2}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 (Methylcholanthrene-induced) of Furth and Kabat | *Pct.* 14.4 | 7.7 | 4.3 | 4.7 | 0.91 | 13.9 | 12.2 | 6.5 | 0.87 | 0.85 | 6.2 | 44 | 3.8 | 8.7 | +4.5 |
| Determinations.....number | | 25 | 25 | 25 | 25 | 27 | 10 | 9 | 10 | 9 | | | | | |
| Tumors.....number | | 9 | 9 | 9 | 9 | 9 | 2 | 2 | 2 | 2 | | | | | |
| 10 of Murphy and Claude | 8.7 | 12.8 | 4.0 | 5.0 | .79 | 22.0 | 19.5 | 10.7 | .96 | .87 | 9.2 | 42 | 5.4 | 13.0 | +12 |
| Determinations.....number | | 7 | 7 | 7 | 7 | 7 | 4 | 4 | 4 | 4 | | | | | |
| Tumors.....number | | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 2 | | | | | |
| 11 of Furth | 10.0 | 7.3 | 4.8 | 5.5 | .88 | 15.3 | 14.9 | 5.9 | 1.04 | .88 | 8.0 | 52 | 4.4 | 8.5 | +4.3 |
| Determinations.....number | | 33 | 33 | 33 | 33 | 26 | 8 | 10 | 7 | 10 | | | | | |
| Tumors.....number | | 10 | 10 | 10 | 10 | 10 | 3 | 3 | 3 | 3 | | | | | |
| 12 of Furth | 10.5 | 6.3 | 2.5 | 3.2 | .68 | 7.6 | 9.6 | 6.3 | 1.10 | .92 | 1.3 | 17 | 1.2 | 6.9 | +1.2 |
| Determinations.....number | | 19 | 17 | 19 | 17 | 18 | 8 | 6 | 8 | 6 | | | | | |
| Tumors.....number | | 7 | 7 | 7 | 7 | 7 | 3 | 3 | 3 | 3 | | | | | |
| 13 of Stubbs and Furth | 10.2 | 8.1 | 3.1 | 3.7 | .86 | 14.9 | 16.1 | 7.1 | 1.03 | .83 | 6.8 | 46 | 5.6 | 12.4 | +7. |
| Determinations.....number | | 27 | 25 | 26 | 24 | 25 | 15 | 12 | 15 | 12 | | | | | |
| Tumors.....number | | 9 | 9 | 9 | 9 | 8 | 5 | 5 | 5 | 5 | | | | | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 of Rous | 9.5 | 10.0 | 1.7 | 2.5 | .67 | 15.8 | 21.5 | 9.1 | 1.26 | .88 | 5.8 | 37 | 7.2 | 19.7 | +10.8 |
| Determinations number | | 19 | 19 | 19 | 18 | 19 | 3 | 4 | 3 | 4 | | | | | |
| Tumors number | | 10 | 10 | 10 | 10 | 10 | 2 | 2 | 2 | 2 | | | | | |
| 1, embryo-cultured: | | | | | | | | | | | | | | | |
| Nodular: | 7.0 | 9.4 | 4.5 | 4.9 | .94 | 16.3 | 16.2 | 7.1 | 1.10 | .86 | 6.9 | 42 | 4.3 | 10.2 | +7.5 |
| Determinations number | | 11 | 11 | 11 | 11 | 6 | 2 | 6 | 2 | 6 | | | | | |
| Materials [2] number | | 4 | 4 | 4 | 4 | 3 | 1 | 2 | 1 | 1 | | | | | |
| Membranous: | 7.7 | 4.8 | 4.7 | 4.2 | 1.18 | 8.1 | 6.7 | 5.3 | 1.34 | 1.10 | 3.3 | 41 | 2.4 | 5.8 | −.3 |
| Determinations number | | 5 | 5 | 5 | 5 | 4 | 1 | 3 | 1 | 3 | | | | | |
| Materials [2] number | | 3 | 3 | 3 | 3 | 2 | 1 | 2 | 1 | 2 | | | | | |
| 1 of Rous: | | | | | | | | | | | | | | | |
| Data of Warburg (*2*) | | 10.0 | | 2.5 | | 15.0 | | | | | 5.0 | 33 | 6.0 | 18.0 | +10.0 |
| Number | | [3] 1 | | [3] 1 | | [3] 1 | | | | | | | | | |
| Data of Murphy and Hawkins (*16*) | | 10.6 | | 3.7 | | 13.8 | | | | | 3.2 | 23 | 3.4 | 11.0 | +6.3 |
| Number | | [3] 4 | | [3] 4 | | [3] 4 | | | | | | | | | |
| 9 of Murphy and Landsteiner: | | | | | | | | | | | | | | | |
| Data of Murphy and Hawkins (*16*) | | 7.3 | | 4.6 | | 11.8 | | | | | 4.5 | 38 | 2.9 | 7.6 | +2.6 |
| Number | | [3] 17 | -- | [3] 17 | | [3] 17 | | | | | | | | | |

[1] Q values based on initial dry-weight values.

[2] See footnote 12, table 1.

[3] Data of Warburg (*2, p. 197*) and Murphy and Hawkins (*16*) were based on original final wet-weight values but are here arbitrarily multiplied by 0.5 to convert to initial wet-weight values. (See table 2.) Similarly corrected, other miscellaneous data for chicken tumor No. 1 of Rous are: Crabtree (*17*), $Q^{N_2}{}_A = 15.3$ (10 determinations), $Q^{O_2}{}_A = 9.8$ (10 determinations), $Q_{O_2} = 6.0$ (10 determinations) (unaccountably variable and probably quite high); and Dickens and Simer (*7*), $Q_{O_2} = 2.6$ (2 determinations), and R. Q. = 0.92 (in Ringer phosphate), the R. Q. being unaffected by insulin or 41° C., but raised to 1.17 by M/45 pyruvate; and Dickens and Greville (*10*), $Q^{N_2}{}_A = 16.5$ (2 determinations), with value about one-half as great with fructose instead of glucose. Dickens and Greville (*10, 13*) obtained with Mill-Hill fowl fibrosarcoma 1, $Q^{N_2}{}_A = 15.4$ (4 determinations,) $Q^{N_2}{}_{LA} = 15.0$ (1 determination), and one-third this value with fructose.

unity, since the histologic examination showed almost no tumor cells, but instead a heavy infiltration of polymorphonuclear leukocytes, together with many embryonal cells. The inclusion of the membranous material in table 4 is thus justified only parenthetically, as is further indicated by the fact that the derived quotients are very different from those of the tumors proper.

The $Q_{LA}/Q_A$ values approximate unity with reasonable closeness, being definitely and slightly lower for the aerobic values and possibly (but doubtfully in reality) higher anaerobically. An anaerobic value higher than unity could mean production of some base as well as lactic acid, but more probably methodology is involved; over the 4-hour periods of observation employed, the pH often decreased by several tenths of a unit, so that lactic acid, especially that produced toward the end of a run, would liberate somewhat less than an equivalent amount of carbon dioxide, with the result that the manometric observations would be correspondingly low, and hence the ratio $Q^{N_2}{}_{LA}/Q^{N_2}{}_A$ high. The manometric determinations did not involve this uncertainty, and there is every reason to believe that $Q^{O_2}{}_{LA}/Q^{O_2}{}_A$ is in fact less than unity, in accordance with other direct chemical measurements by Elliott and coworkers (*36*, *37*, etc.). This means that aerobically the tumors form a certain amount of acid other than lactic acid. For tumors 16, 10, 11, 12, 13, and 1 in table 4 the values for this alactic-acid formation, which may be termed $Q^{O_2}{}_{aLA}=Q^{O_2}{}_A-Q^{O_2}{}_{LA}$, are respectively, 0.93, 1.52, 0.79, 0.45, 1.42, 1.22, 1.61, −0.60, or an average of 0.92. The corresponding $Q^{N_2}{}_{aLA}$ values are 1.73, 0.47, 0.53, −1.02, −0.34, −4.40, −1.50, and −1.70, or an average of −0.78, but as just indicated, these negative anaerobic values are probably methodological, and zero values or even very slightly positive values are more likely.

The derived functions of table 4, namely, the absolute and percent Pasteur effect, Meyerhof oxidation quotient, extent quotient, and the fermentation excess, are, with the possible exception of tumor 12, highly characteristic of malignant tumors. In particular, the fermentation excess is definitely positive, which is the case for almost no nontumor tissue; the percent Pasteur effect is below 50; the Meyerhof oxidation quotient lies between about 3 and 7; and the extent quotient is above 6. The significance of these numerical values has been detailed in recent years by Burk (*5*, *6*).

The results with the chicken tumors throw light on the controversy as to whether the high aerobic glycolysis of malignant tumors is effected by a disturbed respiration in the sense of there being a low Meyerhof oxidation quotient or of there being a low limited respiration diminished in extent. Is aerobic glycolysis due to a deficiency of "effect" or of "extent," in the current terminology? The chicken tumors studied (with the possible exception of the bone osteochondrosarcoma 12) indicate very definitely against the "effect" explanation. The Meyerhof quotients obtained are in the range of 3.8 (or 1.2) to 7.2, a fact which indicates that the Pasteur effect is strongly functioning when one recalls that values above 0 are indicative of its operation. The "extent" explanation, on the other hand, is clearly indicated, in the sense that the respiration values are rather low as compared with other tissues generally ($Qo_2=2.5$—$5.5$ based on initial dry weights), and the anaerobic glycolysis values are so high that the high aerobic glycolysis may be regarded as a residual effect, a result of the fact that even with a high Meyerhof oxidation quotient the respiration cannot by any

means completely prevent glycolysis. The high aerobic glycolysis is best regarded as a residual effect of an abnormally high anaerobic glycolysis and normal respiration operating in tumors as contrasted with the high anaerobic glycolysis and high respiration of growing tissue and relatively lower anaerobic glycolysis and normal respiration of normal tissue.

We do not like to consider that, according to Dickens' view, the medium low R. Q. of malignant tumors represents a "disturbed respiration," since the majority of normal adult tissues also possess R. Q. values well below unity; nor can we adopt (as more than the most occasional exception) the "effect" view added by Warburg to his original "extent" view; the "disturbed respiration" of malignant tumors, if such really exists, consists of merely the low extent of respiration, in accordance with Warburg's first analysis (Burk (*6, pp. 442–445*)), and not of a commonly decreased Meyerhof oxidation quotient ("effect") as well (cf. also *48*).

## METABOLIC-BIOLOGIC CORRELATIONS

### Comparison of Filterable and Non-filterable Tumors

Tumor 16, derived from a chicken that received methylcholanthrene, is transmissible only by cellular grafts, whereas all other tumors studied can be transmitted by cell-free agents. The metabolism of tumor 16 was of essentially the same order as that of the agent-induced tumors, and all the tumors (save possibly No. 12) possess highly characteristic, malignant tumor metabolism. Thus the agent may play a role in tumor genesis or transmissibility but a doubtful direct role in tumor metabolism. The finally formed tumor cells or tissues are quite different from normal cells in possessing their own characteristic metabolism, independent of the mode of origin or transmission. This observation contradicts the opinion that the virus-induced tumors are essentially normal cells driven to continuous multiplication by intracellular ultramicroscopic parasites. Were this opinion correct, the cells of virus-induced tumors might be expected to behave like rapidly regenerating normal cells. Our experience with hepatic tumors of rats (*47, 48*) shows that the metabolism of primary liver carcinomas induced by *p*-dimethylaminoazobenzene (butter yellow) differs markedly from that of liver cells rapidly regenerating as a consequence of surgical removal of 65 percent of the liver (left and median lobes).

### Histologic Examination

The full significance of the desirability of close correlation of metabolic data with the microscopic appearance of the tumors, at least in a certain number of cases, was not fully realized until after this study had been completed. Thus there are not sufficient data to evaluate the metabolic significance of leukocytic, monocytic, and lymphocytic infiltration in the various tumors, the metabolism of avian blood cells being unknown, except by rough analogy. It is known that tumors are "infested" with monocytes (*49*) that are often not readily identified in the usual haematoxylin and eosin preparations employed by us but are quite distinct in sections stained according to Hortega's silver carbonate technique.

This study has led us to conclude that it is somewhat difficult to obtain an almost pure culture of malignant chicken tumor cells or tissues in slices of the size ordinarily employed in manometric studies. In spite of careful dissection, histologic check-up often showed a high percentage of microscopic impurity as areas of de-

generation, necrosis, leukocytic infiltration, and surviving muscle. This emphasizes the difficulty and desirability of attempting to obtain *in vivo* tumor material with a sufficiently high percentage (greater than 80–90 percent) of pure viable tumor of ordinary slice size, and points to the advantageous aspects of the Linderstrom-Lang technique that might be employed, whereby it might be possible to obtain a nearly perfect correlation between pure tissue metabolized and sectioned. Certainly it would be desirable wherever possible to metabolize and section the same (and not aliquot) slices, regardless of amount of material employed in a given determination. This will frequently, as in the present investigation, not be possible, since necessary acidification of the tissue during manometric or chemical analysis precludes adequate histologic procedure. Perhaps the substitution of formalin for acid might overcome this difficulty upon occasion.

Gross variations and atypical behavior in metabolism can often be explained on a morphologic basis. For example, the membranous form of the growth of tumor 1 on the egg membrane, which was grossly regarded as a diffuse tumor infiltration, turned out to be edematous connective tissue containing but few tumor cells with large numbers of polymorphonuclear leukocytes, congested vessels, and slightly hyperplastic epithelium of the membrane. For another example, in studies made in association with J. Langdon Norris, an increase was noted in the anaerobic metabolism of embryonal as compared with adult rat livers. However, microscopic examination showed the presence of large numbers of erythrogenic and leukogenic cells, well-known components of embryonal livers which in themselves almost certainly account for the greatly increased anaerobic glycolysis, a matter which is being investigated further. The hematopoietic elements amounted in the extreme to some 80 percent of the tissue, and the anaerobic glycolysis of the liver was increased from $Q^{N_2}{}_A$ of 1 or less for normal liver to 6 to 8, which is approximately the value given by the nearest homologues studied, nucleated erythroid cells in the bone marrow of the rabbit (*50*), and erythroid hyperplasia of chicken bone marrow induced by acetylphenylhydrazine.

Differences in metabolic values of the different tumors may not be due in the main to differences in metabolism of the respective tumor cells. Sarcoma 16, produced by methylcholanthrene, is composed of densely packed cells with little intercellular substance, while the sarcoma cells of strain 13 are widely separated by large amounts of intercellular substance. Likewise the amount of hemorrhage and necrosis present in tumors of different strains is to some extent characteristic for each strain, although there are considerable individual variations within each strain.

It is our opinion, in fact, that the metabolism of pure tumor cells of all of the strains studied is, on a dry weight basis, probably even more alike than indicated in table 4, when appropriate corrections are made for incidental nontumor material. In drawing the latter generalization, it is necessary to point out that the water content of the chicken tumors is as in most tumors, a definite characteristic to be taken into consideration. As already noted, solid tumor 16, with the highest percent dry weight, shows, as compared with the other tumors, a relatively still greater metabolism (Q values) when calculations are made on a wet-weight basis. Conversely, the embryo-cultured tumor 1, with the lowest percent dry matter would have relatively still lower values on a wet-weight basis. The tumors in table 4 are

listed approximately in order of their percent dry weight, so that on a wet-weight basis the vertical spread of metabolic values would be somewhat widened, not narrowed.

In considering the solidity or denseness of packing of a given tumor, it is important to note that there may be only a moderate correlation between percent dry weight and intensity of staining or number of nuclei, since the unstained elements may contain a high percentage of dry matter. Nevertheless, one must not overlook the occasional desirability of thinking of Q values in terms of wet weight, number or size of nuclei, as well as other chemical criteria (nitrogen content, nucleoprotein content). In our judgment, however, no criterion is so generally useful as the (initial) dry-weight basis.

## SUMMARY AND CONCLUSIONS

An extensive survey has been made of the metabolism, standard Q values, and derived quotients of a wide variety of chicken tumors available in our laboratories. The metabolism of this heretofore little studied class of tumors has been found to be characteristic of the metabolism of malignant animal tumors generally. The chicken tumors possess a high anaerobic and high aerobic lactic acid production, a respiratory quotient well below unity, a marked Pasteur effect, and approximate equivalence of manometrically and chemically determined lactic acid (except, however, for a slight aerobic alactic acid production, $Q^{O_2}{}_{aLA}$ being about 1). Thus the broad conceptions of Warburg and Dickens concerning the rather specific nature of malignant-tumor metabolism are strengthened in generality and extended to a class of tumors in particular which represent, indeed, an upper limit of the generality. A summary table of grand averages, tumor by tumor, is given for all of the metabolic data obtained in this study and also all of the previously reported, relatively scanty chicken tumor metabolism data available.

In addition to the standard metabolic values determined, a number of miscellaneous variables were studied, including temperature, cyanide inhibition, initial and final dry-weight/wet-weight ratios, absence of glucose in the medium, preliminary anaerobiosis, and X-radiation. In confirmation and extension of certain previous work, lactic acid was found to be utilized (by tumor 11) only very slowly under aerobic conditions, and not at all under anaerobic conditions (sensitivity, $Q_{LA}=-0.3$). The sources of error and variability in the different quantitative determinations are discussed at some length in relation to these and to metabolism studies generally. Considerable manometric miscellany is described, and detailed protocols are provided illustrating the use of the Summerson constant-volume differential manometer in obtaining simultaneous aerobic measurements of total net acid production, lactic acid production, carbon dioxide production, oxygen consumption, and respiratory quotient on a given tissue-slice sample.

Tumor 16, induced chemically with methylcholanthrene and never as yet transmitted by a filterable agent, was found to possess much the same metabolic characteristics as the filterable chicken tumors. Tumor 1 of Rous, grown on the chorioallantoic membrane of developing chick embryo, possessed much the same metabolism as when grown on chicks in the usual way, except that the aerobic (but not the anaerobic) glycolysis was lower and the respiratory quotient and rate were definitely higher. The metabolism of all of the tumors, when corrected in the best manner evident, for incidental miscellaneous materials or im-

purities in the specimens metabolized,was quantitatively as well as qualitatively much alike.

The Pasteur effect was as marked in the chicken tumors as in other malignant-tumor or nontumor glycolyzing tissues, The original "extent" theory of Warburg without his later appended "effect" theory suffices to account quantitatively for the Pasteur effect in the chicken tumors, since the Meyerhof oxidation quotients are high (3 to 7, with the exception of the osteochondrosarcoma 12); the anaerobic glycolysis values are so high relative to the respiration that the latter is unable to inhibit completely the glycolysis under aerobic conditions, even with extensive operation of the Pasteur effect (Meyerhof oxidation quotient = 6, whereby the aerobic decrease in fermentation is six times the equivalent oxygen consumption). A common view that the Pasteur effect is small in malignant tumors is thus as unfounded for the chicken tumors as has been indicated earlier (*6*) for malignant tumors generally.

Brief histologic descriptions and photomicrographs of the tumor material employed have been presented illustrating the unavoidable microscopic impurities to be found in tissue slices of the usual size, including intercellular, degenerating, or necrotic tissue, stroma, blood, muscle, leukocytes (monocytes, lymphocytes), etc., that may comprise 10 to 50 percent of the gross tumor even after most careful dissection away from supporting nontumor tissue. Attempts to correlate the metabolic values observed with the gross and microscopic appearance of the chicken tumors and slices revealed that the problem is very complex and that more thorough investigations along these lines, with tumors generally, are needed. Studies with appropriate techniques on material very much smaller than the usual slice size are highly desirable in order to obtain data on essentially homogeneous cells.

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# Studies in Carcinogenesis. XVI. Production of Subcutaneous Tumors in Mice by Miscellaneous Polycyclic Compounds[1]

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Between 1934 and 1939 about 225 compounds were examined in this laboratory for carcinogenic potency in mice. In preceding papers of this series (*1–6*), the results obtained with groups of compounds closely related to 1, 2-benzanthracene, to 1, 2, 5, 6-dibenzanthracene, to 3, 4-benzpyrene, or to 20-methylcholanthrene were recorded. This paper contains a summary of the results obtained with 45 other compounds and completes the reports on the 225 compounds examined in these studies, with the exception of about 40 nitrogen-containing benzene derivatives.

For the most part the compounds[2] were injected as in previous experiments, i. e., in the crystalline form moistened with glycerol for lubrication in the trocar. The properties of the new compounds employed in this work have been described in the papers from the cooperating laboratories of organic chemistry. Unless otherwise specified, only one injection was given, subcutaneously, in the left flank of inbred strains of mice obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. To avoid repetition of the same statement for each experiment, it is pointed out here that 20 mice of the A strain, equally divided between the sexes, were used in each experiment. Statements regarding the number, sex, or strain of mice are given only in the experiments in which the procedure was varied. Solutions in lard, filtered at 38° C., were used in instances in which not enough of the compound was available for administration of the required amount in crystalline form or when low doses were desirved. The animals were usually about 3 or 4 months old at the start of the experiments. They were fed a diet of Purina dog chow and tap water without limitation as in previous experiments.

This paper records the presence or absence of tumors induced at the site of injection. Mention is made of pulmonary tumors in those instances in which they appeared to be more numerous than in comparable untreated mice. The usage of the term "tumor" in referring to the growths reported in this paper is the same as that in a preceding paper (*6*). Pathologic findings other than induced tumors at the site of injection or in the lungs are being reserved for a separate communication. As before, the terms "negative mice" and "negative survivors" designate animals not bearing induced tumors.

Since no tumors were produced with

[1] *This work was carried out in the Office of Cancer nvestigations, United States Public Health Service, then located at the Harvard Medical School, and at the Wolcott Gibbs Memorial Laboratory, Harvard University.*

[2] *Unless otherwise designated, the compounds were synthesized by Fieser and coworkers as part of a cooperative project initiated by the United States Public Health Service in 1934. The preliminary results of these biological experiments were, in some instances, announced in the papers from the laboratories of Professor Fieser (Harvard University) and of Professor Newman (The Ohio State University) in the Journal of the American Chemical Society, which described the syntheses of these compounds.*

most of the compounds reported here, the protocols are given in brief form. Because of the possibility, however, that under different experimental procedures some of these compounds may give rise to tumors, data on dosage and survival are recorded.

## COMPOUNDS RELATED TO 1,2-BENZANTHRACENE

The 11 compounds in this group consisted of 1,2-benzanthracene and 10 related compounds. Carcinogenic potency was exhibited by 3.

*1,2-Benzanthracene (fig. 1, formula I).*—Although many derivatives of 1,2-benzanthracene have been found to be potent carcinogens, the parent hydrocarbon itself was found by the London workers to be almost entirely devoid of potency; only 1 epithelioma was obtained (*7*) in skin-painting experiments with 80 mice, and no tumors were obtained in an experiment, discontinued after a year, in which this compound had been injected subcutaneously into 10 mice. As pointed out previously (*5*), subcutaneous tumors were obtained in this laboratory with a number of hydrocarbons that had previously been reported as giving negative results with the skin-painting technique. Since comparable long-term experiments with this compound were not found in the literature, it was considered desirable to test its effect on subcutaneous tissue. Accordingly, 5 mg. was injected (October 24, 1938) at the beginning of the experiment and again after 6 months. Ten mice survived for 15 months. No tumors were obtained at the injection site by the time the last four mice died at the end of 18 months, although the compound was still present in the subcutaneous tissue a year after the last injection.

The lungs of 2 mice that died after a year contained 6 and 13 tumor nodules, respectively. The maximum number of lung nodules seen at autopsy in the other mice was 4. Andervont and Shimkin (*8*) obtained no increase of pulmonary tumors in strain A mice in a short-term experiment with this compound introduced intravenously. Boyland and Burrows (*9*) gave repeated subcutaneous injections of a colloidal aqueous preparation of 1,2-benzanthracene to 10 rats; no tumors were obtained. This compound thus has almost negligible potency.

*4-Methyl-1,2-benzanthracene (fig. 1, formula II).*—The London group (*7*) had obtained negative results in a skin-painting experiment with the 4-methyl derivative of 1,2-benzanthracene. With the subcutaneous-injection technique, however, rapid tumor production was obtained, as was mentioned in an earlier paper (*3*) in this series.

Only 1 dose, of 2 mg., was injected (March 9, 1938). Ulceration developed at the injection site in several mice during the first 4 months. The first 3 tumors appeared at the end of 3 months; tumors appeared in 2 more mice in the fifth, and in 4 mice in the sixth month, at which time there were 10 negative survivors. Tumors were produced in 12 mice in 8 months, and in a total of 15 of the 20 mice in 11 months. None of the 3 mice negative at 11 months developed tumors by the time the experiment was terminated in the fourteenth month. The lungs were studded with numerous tumor nodules in 1 mouse; in the other mice the number of pulmonary tumor nodules was not unusual.

*4-Methyl-1',2',3',4'-tetrahydro-1,2-benzanthracene (fig. 1, formula III).*—Two milligrams of this compound was injected (March 9, 1938) as the first dose. A tumor was induced at the injection site in 1 mouse in the seventh month. An additional 5-mg. dose was injected after 8

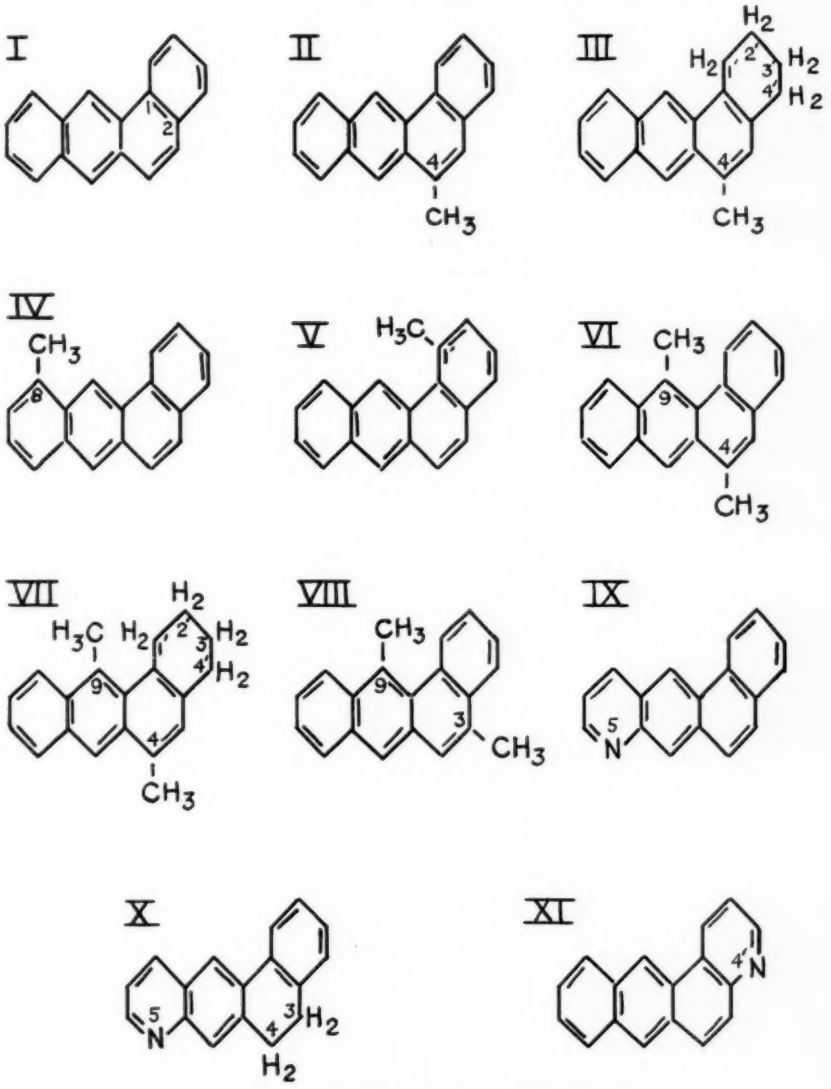


FIGURE 1.—Compounds related to 1,2-benzanthracene.

months and again after 13 months, at which times there were 17 and 11 negative survivors, respectively. No other tumors developed by the time the experiment was terminated in the fifteenth month.

*8-Methyl-1,2-benzanthracene* (*fig. 1, formula IV*).—Three milligrams of 8-methyl-1,2-benzanthracene was injected (July 15, 1938) as the first dose, and 5 mg. was given after 3 and after 9 months. Seven-

teen mice lived for a year. No tumors developed at the injection site by the time the experiment was terminated by killing the 8 survivors in the seventeenth month. The injected compound was seen in the subcutaneous tissue of the 8 mice at autopsy 7 months after the last injection. The lungs contained multiple tumor nodules with a maximum of 15 in 1 mouse, and an average of 5.5 per mouse.

In a skin-painting experiment with 20 mice, the London workers (*10*) obtained with this compound an epithelioma in a mouse that died after 682 days.

*1′-Methyl-1,2-benzanthracene* (*fig. 1, formula V*).—A preliminary report of the negative results obtained with 1′-methyl-1, 2-benzanthracene was given in an earlier paper (*4*) in discussing its structural relationship to 3,4-benzpyrene.

In this experiment, 5 mg. of the compound was injected[3] (July 13, 1937). No tumors were obtained although 12 of the mice survived for 15 months and 2 were still alive when the experiment was terminated in the twenty-first month. This is in agreement with the negative results obtained by the London group (*10*) with this compound.

*4,9-Dimethyl-1,2-benzanthracene* (*fig. 1, formula VI*).—One injection of 2 mg. was given (October 19, 1938). Ulceration at the site of injection developed in 8 of the 20 mice during the first few months. A tumor appeared in 1 mouse at the end of the third month, in another in the fourth, and in 3 more in the fifth month. Tumors were induced in 9 of the mice in 7 months at which time there were 5 negative survivors. None of these developed tumors by the time the last 4 mice were killed in the twelfth month.

*4,9-Dimethyl-1′,2′,3′,4′-tetrahydro-1,2-benzanthracene* (*fig. 1, formula VII*).—The first injection (March 9, 1938), which consisted of 2 mg., was followed 8 and 12 months later by injections of 5 mg. each. Seventeen of the mice were alive at the end of a year. No tumors developed in any of the mice by the time the experiment was terminated in the sixteenth month.

*3,9-Dimethyl-1,2-benzanthracene* (*fig. 1, formula VIII*).—Two milligrams was injected (March 16, 1939). No induced tumors had appeared by the time the experiment was terminated in the thirteenth month by killing the 10 survivors.

The 9-methyl derivative, in this laboratory, and the 3-methyl derivative, in London, had been found to produce sarcomas when injected subcutaneously into mice. Yet when both groups were introduced into the same molecule, the 3,9-dimethyl compound was found to be inactive. This is another instance in which a "summation effect" was not obtained (*5*).

*5-Aza-1,2-benzanthracene* (*fig. 1, formula IX*).—Interesting results have been obtained in London with analogues of the carcinogenic hydrocarbons in which a nitrogen atom occupies the position of a ring carbon. Newer results and references to earlier findings are given in a recent paper (*10*). In these compounds the nitrogen is in the meso position. A group of compounds containing nitrogen in one of the end rings of structures related to 1,2-benzanthracene, obtained by Dr. Voegtlin from Dr. Mosettig, of the Drug Addiction Laboratory of the National Research Council, has been examined in this laboratory. Analogous compounds are also being investigated by Fieser and Hershberg (*11*) whose nomenclature for such compounds is being followed here.

Naptho(1,2-g)quinoline, synthesized by Mosettig and Krueger (*12*), may be con-

[3] *By Arnold M. Seligman, Harvard Medical School.*

sidered an analogue of 1,2-benzanthracene with nitrogen replacing carbon at the 5-position and may, accordingly, be designated as 5-aza-1,2-benzanthracene. Two milligrams was given at the first injection (October 18, 1938), and 5 mg. was given 6 months later. At this time there were 19 survivors. An epidemic infection by *Bacillus piliformis*, however, brought this experiment to a close in the tenth month. No induced tumors were noted.

*3,4-Dihydro-5-aza-1,2-benzanthracene* (*fig. 1, formula X*).—The 3, 4-dihydro derivative of compound IX, designated as 5,6-dihydronaphtho(1,2-g)quinoline by Mosettig and Krueger (*12*) who synthesized it, was injected in the same dosage and at the same times as the preceding compound. Thirteen mice lived for 9 months; the six sickly survivors were killed in the twelfth month. No tumors were induced.

*4′-Aza-1,2-benzanthracene* (*fig. 1, formula XI*).—The compound designated by earlier workers as β-anthraquinoline was renamed 4′-aza-1,2-benzanthracene by Fieser and Hershberg (*11*) for "convenience in correlating heterocycles of this type with the hydrocarbons which they resemble." Thirty mice of the C3H strain (20 males and 10 females) received (May 8, 1939) 5 mg. of this compound [4] subcutaneously. The injection was repeated 3 months later.

Twelve mice lived for 9 months. The experiment was terminated in the eleventh month by an epidemic infection. No tumors developed at the site of injection in any of the mice, and no gross renal tumors were noted although Sempronj and Morelli (*14*) reported having obtained tumors in the kidneys of 2 of 11 rats in 11 months. Joseph (*15*) obtained negative results in mice with this compound, with both the skin-painting and subcutaneous-injection techniques in experiments which did not go beyond 7 months.

[4] *The specimen used was prepared by Dr. J. L. Hartwell according to Graebe's method* (13). *After chromatographic absorption, the melting point was 172.9°–173.4° corrected.*

## COMPOUNDS RELATED TO 3,4-BENZPHENANTHRENE

In view of the interesting results obtained in Kennaway's laboratory with 3,4-benzphenanthrene and with its 2-methyl derivative, six compounds of this type were examined for potency in addition to the 6,7-dimethyl derivative which had been found earlier (*3*) to give negative results.

*3,4-Benzphenanthrene* (*fig. 2, formula XII*).—A specimen of 3,4-benzphenanthrene, synthesized by Newman and Joshel (*16*), was injected (October 19, 1938) in a dose of 2 mg. In the eighth month an epidemic infection broke out, and 8 mice were killed to prevent its spread to other mice. The experiment was terminated in the twelfth month when the last three survivors were killed. No induced tumors were obtained. According to the London workers (*17*), this compound produced skin tumors "after a prolonged latent period." They (*10*) obtained negative results in 10 mice in which a solution of the hydrocarbon in sesame oil had been injected subcutaneously; by the same technique a sarcoma was produced in 1 of 6 rats in about 17 months.

*2-Methyl-3,4-benzphenathrene* (*fig. 2, formula XIII*).—According to the London workers (*17*), the 2-methyl derivative of 3,4-benzphenanthrene produced skin tumors so rapidly as to suggest "an activity approaching that of methylcholanthrene."

However, by the subcutaneous-injection technique, negative results were obtained with a specimen synthesized by Newman and Joshel (*18*). Two milligrams was given (October 19, 1938) at the first injection, and 1 mg. was given 3 months later. Fourteen mice lived for 9 months

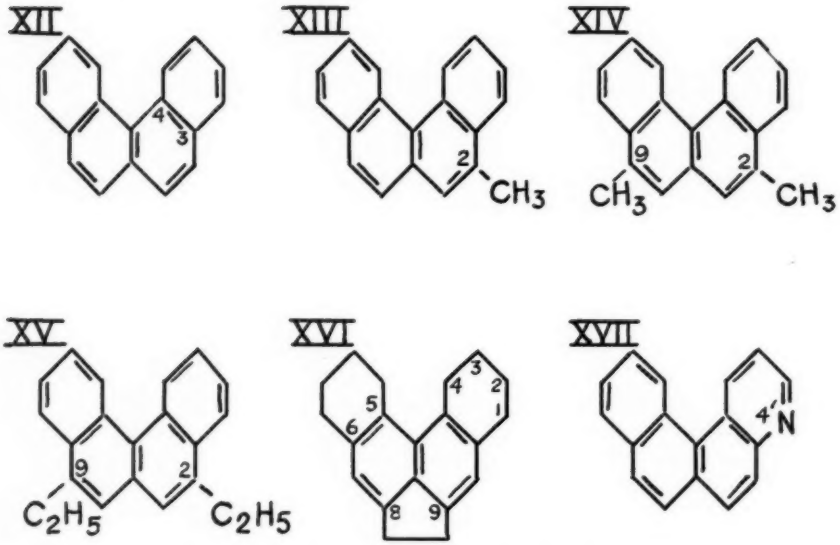


FIGURE 2.—Compounds related to 3,4-benzphenanthrene.

The animals began dying off rapidly in the tenth month, and the five negative survivors were killed in the twelfth month.

No tumors were induced in this experiment, although with the skin-painting technique the London workers obtained tumors in 9 of 20 mice in 9 months and although in the subcutaneous-injection experiment of Andervont and Shimkin (*8*) tumors were obtained in 4 of 18 mice. The latter workers also found that it possessed only weakly carcinogenic properties for the lungs of strain A mice following subcutaneous or intravenous injection. These various experiments show that its potency does not approach that of 20-methylcholanthrene when administered subcutaneously or intravenously.

*2,9-Dimethyl-3,4-benzphenanthrene* (*fig. 2, formula XIV*).—The entire small specimen of 2,9-dimethyl-3,4-benzphenanthrene, synthesized by Newman and Joshel (*16*), was dissolved in lard filtrate in a concentration of 5 mg. per cubic centimeter. The injection of 0.2 cc., given at the start of the experiment (October 29, 1937), was repeated after 4 and after 5 months. There were 14, 10, and 5 survivors at 12, 15, and 18 months, respectively. No tumors were induced by the time the experiment ended in the twentieth month.

*2,9-Diethyl-3,4-benzphenanthrene* (*fig. 2, formula XV*).—Since only a small amount of the 2,9-diethyl derivative of 3,4-benzphenanthrene, synthesized by Newman and Joshel (*16*), was available, it was injected (December 17, 1937) into only 10 mice; the injection of 2 mg. was repeated 4 months later. Seven of the ten mice survived for 9 months. The experiment was terminated after 11 months by an epidemic infection. No induced tumors were noted.

*5,6-Tetramethylene-1,2,3,4-tetrahydro-8,9-acephenanthrene* (*fig. 2, formula XVI*).—This compound was injected (March 16, 1937) into 10 C3H male mice in a dose of 5 mg., which was repeated after 6, 9

and 13 months. All of the mice were alive at the end of a year. No tumors were induced at the injection site in this experiment which was terminated in the fifteenth month when the 7 survivors were killed. Hepatomas, probably spontaneous in origin, were observed in 5 of these C3H mice.

*4'-Aza-3,4-benzphenanthrene* (*fig. 2, formula XVII*).—This nitrogen-containing analogue of 3,4-benzphenanthrene, designated naphtho (1, 2-f) quinoline by Mosettig and Krueger (*12*) who synthesized it, was given (October 18, 1938) in a dose of 2 mg.; 6 months later 5 mg. more was given. Twelve mice lived for 7 months. No tumors had appeared by the time the experiment was terminated in the twelfth month by killing the 3 survivors. Joseph (*15*) obtained negative results with this compound, which he designated 3(N),4-pyridinophenanthrene, in skin-painting and subcutaneous-injection experiments in mice lasting about 7 months.

## COMPOUNDS RELATED TO PHENANTHRENE

Nine compounds related to phenanthrene were examined for potency.

*2,3,?-Trimethylphenanthrene* (*fig. 3, formula XVIII*).—A trimethylphenanthrene with a melting point of 148°–149°, synthesized by Fieser and Holmes (*19*), in which the points of attachment of the 3-methyl groups were still undetermined, was among the compounds examined for potency. Holmes (*20*) stated that it was possibly 2,3,?-trimethylphenanthrene. The entire small specimen was dissolved in lard filtrate and injected (December 10, 1937) into 10 mice in a dose of 0.5 mg. contained in 0.25 cc. of lard. Six of the ten mice were alive at the end of a year. No induced tumors were noted by the time the last 4 survivors were killed in the seventeenth month.

*3,4-Disodium phenanthrene dicarboxylate* (*fig. 3, formula XIX*).—The disodium salt of 3,4-phenanthrene dicarboxylate was injected (June 30, 1936) into 10 mice in a dose of 10 mg. which was repeated 2 months later; another injection, of 5 mg., was given after 4 months. The 7 survivors were killed at the end of a year. No tumors had developed at the injection site, but in one of the mice killed at the end of the experiment a renal epithelial tumor was seen.

Several additional experiments were therefore set up to ascertain whether renal tumors would again be obtained. The compound, in a dose of 10 mg., was injected (February 19, 1938) into 10 males and 10 females of the A strain, and into 10 C3H males; 6 more injections, of 5 mg. each, of the crystalline compound were given during the first 2 months. Then the compound was given in the form of an aqueous solution, adjusted to neutrality, containing 5 mg. per cubic centimeter; 60 injections of 1 or 2 mg. each were given during the next 7 months until a total of 65 mg. was given in the form of an aqueous solution. Of these 30 mice, 12 survived for 9 months and 6 lived for a year. No gross tumors of the kidney or at the injection site were observed in this experiment in which the last mouse died in the fourteenth month.

In the third experiment five males and five females were given (October 8, 1938) an aqueous solution of this salt in lieu of drinking water. The solution contained 0.33 mg. of the compound per cubic centimeter and was neutralized with HCl. After a month 0.01 millimole phosphate per liter was added as buffer. The average intake of the compound was about 2 mg. per mouse per day. Seven of the ten mice lived for 9 months. The four survivors were killed at the end of a year. No gross tumors were seen in the kidneys.

*Phenanthrene-3,4-dicarboxylic acid monohy-*

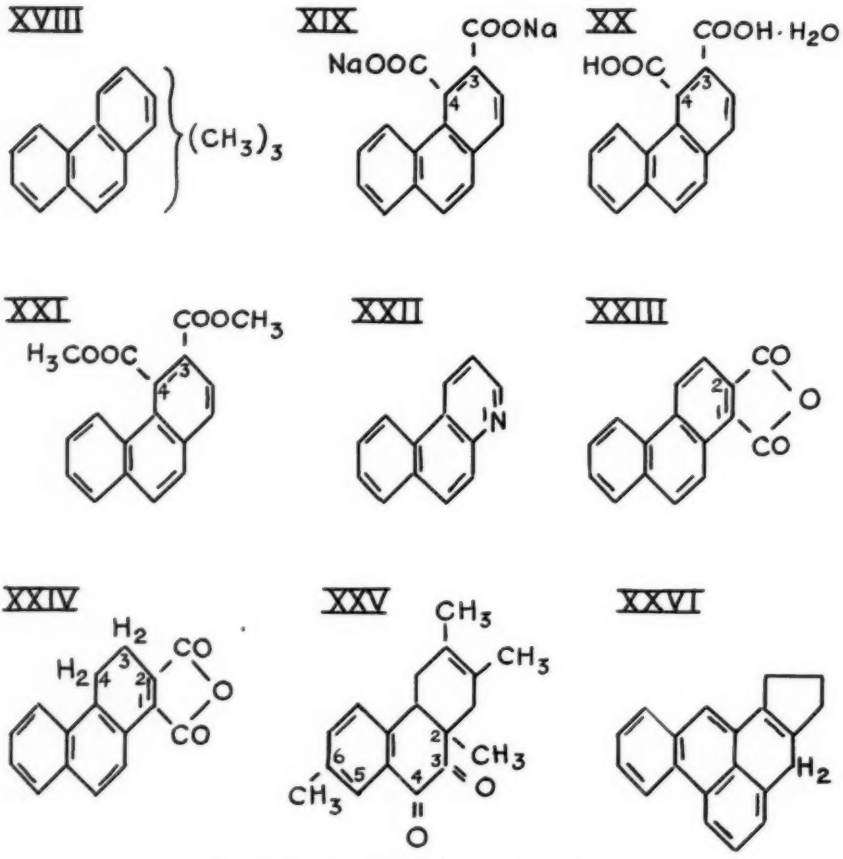


FIGURE 3.—Compounds related to phenanthrene.

*drate* (*fig. 3, formula XX*).—The monohydrate of the free acid was injected (October 22, 1938) in a dose of 5 mg. which was repeated after 3, 6, and 9 months. Survivors at 9 and 12 months were 15 and 6, respectively. No induced tumors were seen by the time the last 2 mice were killed in the fourteenth month.

*3,4-Dicarbomethoxy phenanthrene* (*fig. 3, formula XXI*).—The dimethyl ester was injected (October 24, 1938) into 20 male mice in a dose of 2 mg., and additional injections of 5 mg. each were given 3, 6, and 9 months later. Sixteen of the mice lived for a year. No induced tumors had appeared by the time the experiment ended in 14 months.

*1-Aza-phenanthrene* (*fig. 3, formula XXII*).—To bring out the relationship to phenanthrene, 5,6-benzoquinoline may be designated as 1-aza-phenanthrene. A commercial specimen, purified by Dr. J. L. Hartwell, with a melting point of 90.5°–91.5° corrected, was injected (May 19, 1939) into 30 C3H male mice in a dose of 2 mg. which was repeated after 3 months. After

9 and 12 months, there were 24 and 5 survivors, respectively. No tumors were seen in the kidneys or in the subcutaneous tissue in this experiment which lasted into the seventeenth month.

*Phenanthrene-1,2-dicarboxylic anhydride (fig. 3, formula XXIII).*—The anhydride of the 1,2-dicarboxylic acid derivative of phenanthrene was injected (July 2, 1935) into 12 mice in a dose of 10 mg. which was repeated after 2, 9, and 13 months. There were 11 and 5 survivors after 9 and 12 months, respectively. The experiment lasted 18 months. No induced tumors were observed.

*3,4-Dihydrophenanthrene-1,2-dicarboxylic anhydride (fig. 3, formula XXIV).*—The 3, 4-dihydro derivative of compound XXIII was injected (July 2, 1935) into 21 mice in a dose of 10 mg.: 3 more injections of 10 mg. each and 2 of 5 mg. each were given in the course of this experiment which lasted 19 months. Fifteen of the mice lived for a year. No tumors were induced.

*2, 6-Dimethyl-3, 4-naphthoquinone-2,3-dimethylbutadiene (fig. 3, formula XXV).*—This compound, which is a derivative of a tetramethyl phenanthrene and which is related to compounds (*21*) possessing estrogenic activity, was injected (September 19, 1935) into 20 female mice. The initial dose was 10 mg.; 2 other injections of 5 mg. each were given 3 and 6 months later. There were 18 and 13 survivors at 12 and 15 months, respectively. No tumors were induced in this experiment which lasted for 20 months.

*$\Delta^3$-Dehydro-3, 4-trimethylene-isobenzanthrene-2 (fig. 3, formula XXVI).*—Five milligrams was injected (February 12, 1937) into 10 strain M male mice; the injection was repeated after 5, 10, and 14 months. At the end of a year a sarcoma developed at the injection site in 1 animal, while the other 7 survivors were negative. In this experiment, which lasted for 17 months, tumors were obtained at the injection site in 6 of the 10 mice.

## COMPOUNDS RELATED TO PYRENE, CHRYSENE, AND TRIPHENYLENE

In addition to the previously reported compounds of these types, eight others were examined for potency.

*Pyrene (fig. 4, formula XXVII).*—Pyrene, the parent hydrocarbon of 3, 4-benzpyrene, was injected (January 5, 1939) into 30 mice in a dose of 10 mg., which was repeated after 4 months. There were 23 mice alive at the end of a year, 13 at 15 months, and 9 at 18 months when the survivors were killed. No malignant tumors were induced, which is in agreement with the results of the skin-painting experiments of Bloch (*22*) and of the London workers (*7*, *10*). The average number of pulmonary tumors in the mice at 18 months was only 1.6.

*1′-Aza-3,4-benzpyrene (fig. 4, formula XXVIII).*—For ready comparison with 3,4-benzpyrene, the compound 3(N), 4-pyridinopyrene, synthesized by Vollmann, Becker, Corell, and Streeck (*23*), may be designated 1′-aza-3,4-benzpyrene. These authors suggested that it might be of interest, in view of its structural relationship to 3, 4-benzpyrene, to test it for carcinogenic potency. A sample of this compound, procured by Professor Fieser, was injected (October 18, 1938) in a dose of 2 mg., followed 6 months later by one of 5 mg. The experiment was brought to an end in the ninth month by an epidemic infection. No induced tumors were observed in this period.

*Chrysene (fig. 4, formula XXIX).*—Chrysene was injected (January 5, 1939) into 30 mice in a dose of 10 mg., which was repeated after 4 months. There were 27 and 13 survivors after 9 and 12 months, respectively. The experiment was ended

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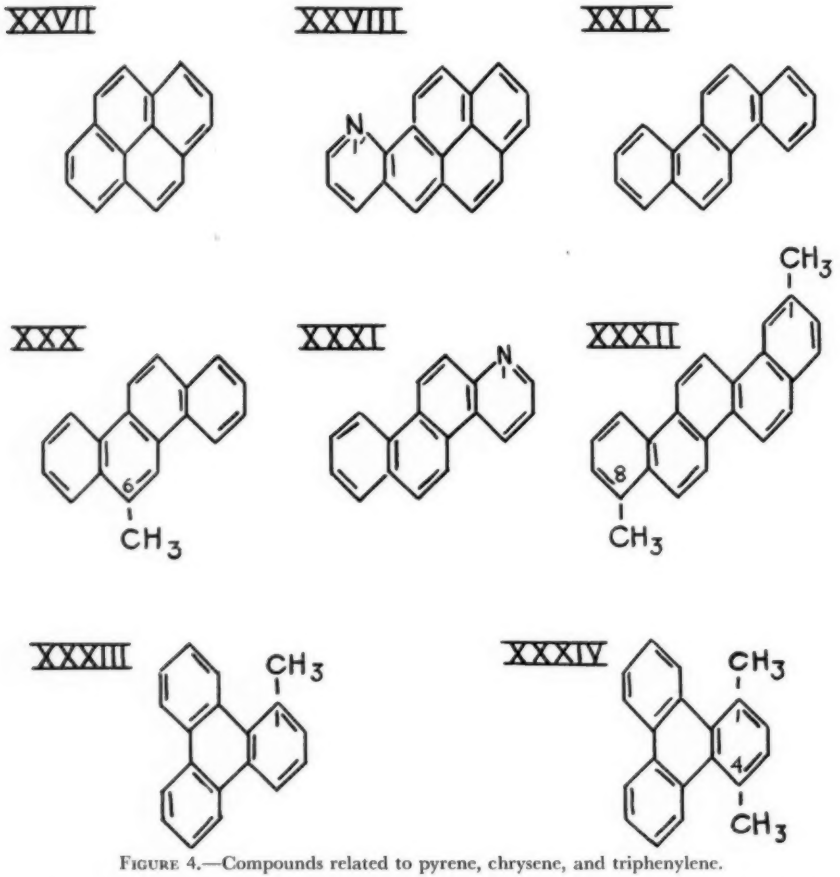


FIGURE 4.—Compounds related to pyrene, chrysene, and triphenylene.

in the fifteenth month at which time 6 mice were still alive. No induced tumors were observed. No epitheliomas were obtained with this compound in the skin-painting experiments of the London workers (*7*, *17*) or of Schürch and Winterstein (*24*). Barry and Cook (*25*), upon injecting a lard solution of chrysene extracted from coal tar, obtained no tumors in mice but did obtain tumors in 4 of 10 rats. They raised the question as to whether their positive results and those previously reported from Twort's laboratory were due to some impurity in the chrysene isolated from coal tar. It is also conceivable that the lard may have contributed to the induction of the subcutaneous tumors in the rats since the experiment of Barry and Cook with mice and that of Boyland and Burrows (*9*) with an aqueous dispersion of chrysene injected into rats were negative, and since the London workers (*26*) have shown that lard alone may induce sarcomas in rats. Similarly, the positive results with mice, obtained with chrysene in Twort's laboratory, may have been due in large part to the oleic acid employed, particularly since Twort and Twort (*27*)

have reported that oleic acid has a "sensitising" effect. In any case, neither in mice nor in rats have tumors been induced with synthetic chrysene.

*6-Methylchrysene* (*fig. 4, formula XXX*).—Two milligrams of 6-methylchrysene, synthesized by Newman (*28*), was injected (October 18, 1938); the dose was repeated after 6 months. Ulceration at the injection site occurred in 3 of the 20 mice during the first few months. At 9 months, 15 of the mice were alive. A sarcoma developed at the injection site in 1 animal in the tenth month. The crystalline compound was seen in the interior of the tumor at autopsy. There were 9 survivors after a year, and the last mouse died in the sixteenth month. No other induced tumors were noted.

*1-Aza-chrysene* (*fig. 4, formula XXXI.*)—A specimen of naptho(2,1-f)quinoline, here called 1-aza-chrysene, was obtained from Mosettig and Krueger (*12*). Two milligrams was injected (October 18, 1938), and 5 mg. was given 6 months later. No induced tumors were noted by the time the seven survivors were killed in the twelfth month.

*1,8-Dimethylpicene* (*fig. 4, formula XXXII*).—Two milligrams of 1,8-dimethylpicene, synthesized by Drake and Howard (*29*), of the University of Maryland, was given at the first injection (February 11, 1938), and 5 mg. was injected 9 months later. Only 10 mice were employed because of the small amount of material available. No induced tumors were observed by the time the 3 survivors were killed in the fourteenth month.

*1-Methyltriphenylene* (*fig. 4, formula XXXIII*).—Five milligrams of 1-methyltriphenylene was injected (January 9, 1939) at the start of the experiment, and again 4 months later. At the end of 15 months the 12 survivors were killed. No induced tumors were observed.

*1,4-Dimethyltriphenylene* (*fig. 4, formula XXXIV*).—One injection of 5 mg. was given (April 17, 1939). No induced tumors were noted in this experiment which was terminated in the fifteenth month when the 12 survivors were killed.

## INDOLE DERIVATIVES

The literature [5] contains allegations that indole, a constituent of feces, possesses tumor-producing properties. Six compounds containing the indole structure were injected, including three heteroauxins which act as plant growth hormones.

*Indole* (*fig. 5, formula XXXV*).—A commercial sample of crystalline indole in a dose of about 10 mg. and 0.5 cc. of a 5-percent solution of indole in lard filtrate proved lethal to mice. They tolerated 0.25 cc. of a 1-percent solution which was injected (December 19, 1934) into 10 female heterozygous albino mice and 12 strain M mice. Twelve injections were given during a 2-year period; the total amount of indole given was 30 mg. for the longest lived mice. Eighteen mice lived a year, and 9 survived 18 months. The last mouse died in the twenty-fifth month. No induced tumors were seen.

*Skatole* (*fig. 5, formula XXXVI*).—Skatole, another fecal constituent, has been reported (*31, 32*) as acting like a growth-promoting hormone in plants. A commercial sample, in a dose of about 10 mg., proved lethal when the crystals were moistened with either glycerol or lard filtrate or were introduced without vehicle. The mice tolerated 0.25 cc. of a 5-percent solution in lard filtrate. The first injection (January 11, 1935) was followed by 12 injections over a period of 22 months; the total amount which the longest lived mouse received was about 160 mg. Female mice were employed in

[5] *Hartwell* (30) *has summarized the literature on indole.*

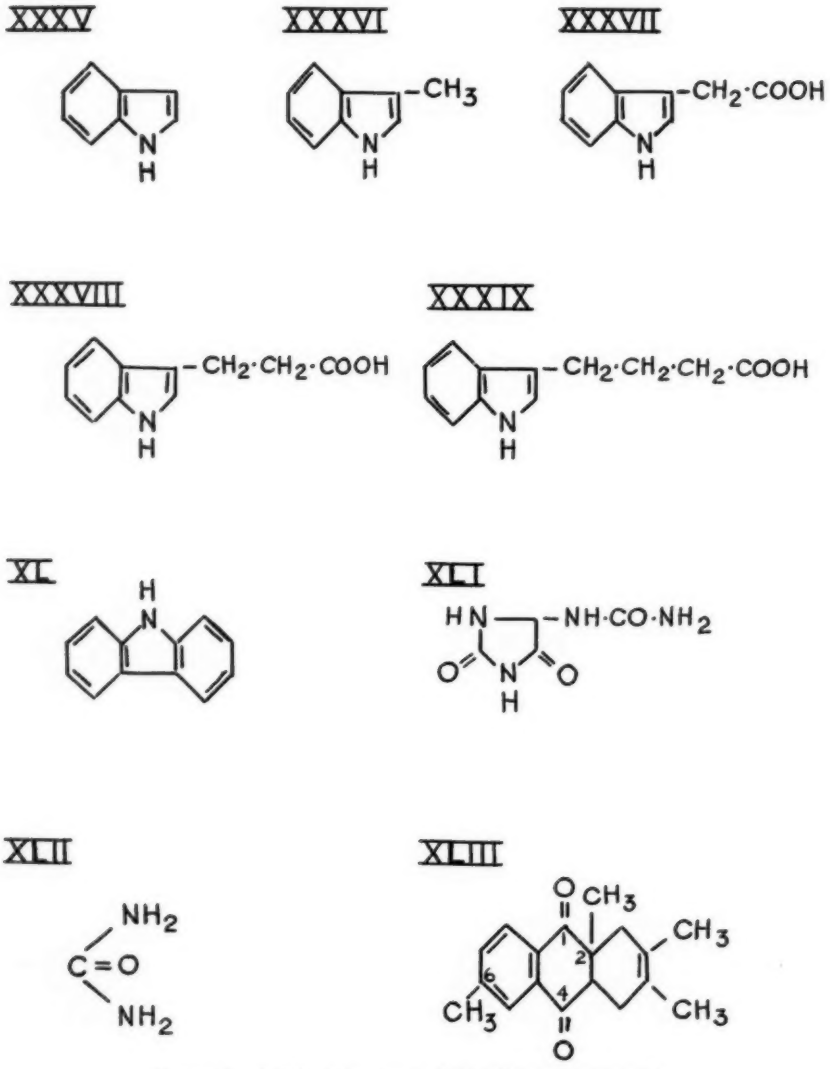


FIGURE 5.—Indole derivatives and miscellaneous compounds.

this experiment; 8 were of the dba strain and 20 were heterozygous albinos. Fifteen survived for 1 year and 10 for 18 months. The last 2 survivors were killed in the twenty-sixth month. No induced tumors were observed.

*Indole-3-acetic acid (fig. 5, formula XXXVII).*– A commercial sample of this heteroauxin was injected (August 25, 1936) into 20 female dba mice in a dose of 10 mg. During the course of 14 months 21 injections totaling 175 mg. were given. Four-

teen mice survived for a year, and 7 for 18 months. The experiment was terminated in the nineteenth month. No induced tumors were obtained.

*β-(Indole-3)-propionic acid (fig. 5 formula XXXVIII).*—A commercial sample of this heteroauxin was injected (August 25, 1936) into male mice in a dose of 10 mg. The number of injections and dosage were the same as for compound XXXVII. At 10 months 19 mice were alive, but during the eleventh month 10 of them died or were killed because of an epidemic infection. Four survived for 18 months. The experiment ended in the nineteenth month No induced tumors were observed.

*γ-(Indole-3)n-butyric acid (fig. 5, formula XXXIX).*—A commercial sample of the third heteroauxin was injected (August 25, 1936) into 20 female dba mice in the same number and size of doses as with the two preceding compounds. Ten of the mice survived for a year, and 4 were alive when the experiment was terminated after 16 months. No induced tumors were observed.

*Carbazole (fig. 5, formula XL).*—About 10 mg. of carbazole was injected (October 26, 1935) into 10 male mice. Five additional injections were given during the course of this experiment in which a total of about 60 mg. was injected. All 10 of the mice lived for a year, and 7 lived for 15 months. The 4 survivors were killed in the nineteenth month. No induced tumors were obtained, which is in agreement with the negative results reported by Kennaway (*33*) and by Schürch and Winterstein (*24*) in skin-painting experiments lasting 9 months. Although Maisin and coworkers (*34*) obtained a sebaceous carcinoma in 1 of 30 mice painted with this compound, they stated that their specimen was not pure; moreover, they used lanolin as a vehicle for the carbazole. Thus far there is no clean-cut evidence that carbazole possesses carcinogenic potency.

## MISCELLANEOUS COMPOUNDS

*Allantoin (fig. 5, formula XLI).*—Because of the reported effect of allantoin on wound healing (*35*), it was tested for carcinogenic potency. Ten milligrams of a commercial specimen was injected (June 30, 1936) into 20 strain A and 3 dba male mice. Nineteen injections totaling 180 mg. were given in the course of 15 months. Seventeen mice lived for a year, and 7 lived for 15 months. The 2 survivors were killed at 18 months. No induced tumors were seen.

*Urea (fig. 5, formula XLII).*—Since urea is a component part of the allantoin molecule, it was injected (August 24, 1936) into 20 strain A and 10 C57 Black male mice. The initial dose of 10 mg. was progressively increased to a level of 50 mg. Repeated injections were given over a period of 11 months until a total of 800 mg. was given. Nineteen mice lived a year, and 5 survivors were killed after 15 months. No induced tumors were observed.

*2,6-Dimethyl-1,4-naphthoquinone-2,3-dimethyl butadiene (fig. 5, formula XLIII).*—Ten milligrams was injected (September 19, 1935) into 22 female mice, and the injection was repeated 3 times during the next 8 months. Thirteen of the mice were still alive when the experiment was terminated after 18 months. No induced tumors were observed.

*Cystine.*—In view of Hammett's statements (*36*) that cystine and related sulfur-containing compounds play an important role in proliferation, and therefore in malignant growth, a commercial sample of *l*-cystine was injected (December 13,1934) into 33 mice, consisting of 7 dba males, 14 heterozygous albinos (males and females), and 12 strain A females. Eleven injections totaling about 110 mg. were

given over a period of 10 months. Fifteen mice lived a year, and 8 lived 18 months. The experiment was terminated after 22 months. No induced tumors were obtained.

*Silica.*—As a further check on the relationship of nonspecific "chronic irritation" to tumor genesis, 10 mg. of powdered silica, suspended in 0.25 cc. of a 0.9-percent solution of NaCl, was injected (January 16, 1937) intraperitoneally into 20 male C57 Black mice, and the injection repeated 5 months later. Nineteen mice lived a year, and 10 were alive when the experiment was terminated after 15 months. No induced tumors were obtained.

## DISCUSSION

The 45 compounds reported in this paper, injected into about 1,000 mice, gave negative results in most instances. Tumors were induced by only 5. Together with the results published previously from this laboratory, a total of 181 compounds was examined; of these, 60 exhibited carcinogenic potency.

In bringing to a close in this laboratory, for the time being at least, the investigation of the carcinogenic potency of groups of polycyclic compounds related more or less closely to 1,2-benzanthracene, it may be useful to survey the findings from this and other laboratories and to consider some of their implications.

First of all, it is desirable to bear in mind that the statement to the effect that given compounds are noncarcinogenic does not necessarily mean that they are incapable of inducing malignant tumors; it means only that the compounds did not give rise to tumors under the conditions of certain experiments of restricted scope. For example, the hundreds of such compounds examined for potency in Kennaway's laboratory in London and in this laboratory have, with few exceptions, been tested in mice only. The small number which were tested in several species of animals have given results which make clear that there are pronounced differences in the response of different species to the action of compounds carcinogenic in the case of the mouse. Moreover, there is evidence that some compounds which were negative in the mouse can induce tumors in other species *(30)*.

Furthermore, in the mouse itself, it is now abundantly evident that different tissues respond differently to the same compound. Some compounds have given negative results when applied to the skin but have been quite active when injected subcutaneously. With other compounds both techniques have given positive results but have brought out pronounced differences in the response of the skin and of the subcutaneous tissue. Compounds that are potent for the skin and subcutaneous tissue were found to be inactive when introduced directly into the liver (*37*). The susceptibility of the lungs (*8*) does not necessarily parallel that of the skin or the subcutaneous tissue.

The mode of administration may also influence the results markedly. The dose may be too high as well as too low, and the most effective dose for one compound is not necessarily the same as that for another closely related one. The solvent or vehicle may affect the results profoundly. Media of complex nature, such as lard or sesame oil, may contain anticarcinogens or co-carcinogens (*38*), and even chemically homogeneous vehicles may give different results (*39*) with the same dose of the same carcinogen administered to the same tissue of the mouse.

Moreover, the sex of the animal is not without influence on the results (*38, 40*). It is also conceivable that the life span of the mouse is a factor. Since some com-

pounds which were negative during the first year gave rise to tumors during the second year of the experiment, it is possible that some compounds which gave negative results in the mouse might give positive results if its life span were longer. Diet, too, may be an important factor (*41*).

For these various reasons the considerations, stated in an earlier paper (*3*) in a discussion of the concept "average time" of the latent period of carcinogens, are also applicable here, viz, the term "carcinogenic potency" as used in these studies is not to be considered as an invariable property inherent in a compound but is merely a summary of the results of particular experiments and is valid only for animals of the species, strain, sex, age, diet, etc., of the particular animal employed, as well as for the dose, menstruum, mode and site of application, etc., of the compound in question. Thus, the compounds examined by the London group were usually applied to the skin of heterozygous mice in the form of a 0.3-percent solution in benzene; in this laboratory the compounds were usually injected subcutaneously into strain A mice in doses of 1 to 10 mg. of the crystalline compound. Conclusions regarding the potency of any given compounds should therefore be interpreted in the light of the data upon which they are based.

On the other hand, these restrictions of interpretation do not mean that there is no specificity of action and that every substance can be shown to be carcinogenic when the right conditions are present. Quite the contrary. The findings show clearly that under given experimental conditions there are most marked differences in potency exhibited by compounds of very closely related structures. The fact that compounds of types other than the polycyclic compounds are also capable of inducing tumors does not detract from the specificity existing among compounds of the latter category. In fact, an entirely analogous specificity is shown by closely related compounds of the type of 2-amino-5-azotoluene (*42*, *43*).

In one sense, this line of inquiry has been disappointing. It is true that it has yielded results which have been, and still are useful in the study of many aspects of experimental cancer. It has provided tools which are being put to many uses, but it has not realized the hope held, perhaps overoptimistically, in some quarters that it would shed light on the genesis of spontaneous tumors.

It has, indeed, clarified the problem of the genesis of certain types of occupational neoplasms, but it has left still unanswered the question of the origin of other human tumors. For one thing, the fact that so many compounds possess carcinogenic potency has been disappointing to some. Perhaps it would have been simpler if all the compounds had proven negative except one. But inasmuch as cancer may be considered not a single disease but a group of disorders with some common characteristics, it might be more reasonable to expect that a considerable number of agents might incite these various types of malignant growths rather than to expect that a single compound would be the cause of all neoplasms.

Apart from the matter of the multiplicity of the potent compounds is the question of the relationship, if any, of such compounds to those that may be responsible for the genesis of spontaneous tumors. At the present time, the only naturally occurring compounds which have been proven to be capable of giving rise to tumors when administered to appropriate animals, are the female sex hormones. As is now well known, certain of the synthetic carcinogens bear interesting structural relationships to these sex hor-

mones. It is still an open question, however, as to whether malignant tumors may be induced by other compounds of biologic origin. The present situation regarding desoxycholic acid has been discussed in a recent paper (*6*). The reported production of tumors by body fluids and tissue extracts is of great interest in this connection, but no information is as yet available as to the identity of the compounds responsible.

The investigations on the relationship of chemical structure to carcinogenic potency in the case of the synthetic carcinogens have, however, given strong support to the hypothesis that chemical compounds may be responsible for initiating spontaneous tumors. This working hypothesis is being employed in many current approaches to this problem. For one thing, extracts of body tissues and of body fluids are being examined for carcinogenic potency; the results reported by Schabad (*44*) and subsequent workers have given added impetus to this approach. On the synthetic side, compounds more closely related to biologically occurring polycyclic compounds than are the derivatives of the benzanthracene hydrocarbons are being synthesized and examined. In this connection, attention is being paid to partially hydrogenated derivatives and to those with polar substituents such as occur in biologic compounds. Although such derivatives of the benzanthracene type have been negative for the most part, we have found a number of them to possess activity. If there are biologically occurring compounds with carcinogenic potency, they might perhaps be expected to be partially hydrogenated and to have polar substituents rather than to be completely aromatic hydrocarbons. Some work along these two lines is now in progress here, in cooperation with chemists at several universities.

Another possibility is the oral introduction of carcinogens inadvertently contained in the diet. The claims of Rowntree and his associates (*45*) and of Roffo (*46*) have not been substantiated to date. Nevertheless, this hypothesis is still sufficiently attractive to warrant further investigation.

The production of experimental tumors by exposure of animals to ultraviolet light and to sunlight is another approach which is being actively studied in several laboratories. Here, too, the hypothesis of a chemical carcinogen is fruitful in suggesting further experiments. Irradiation *in vitro* of skin, skin lipoids,[6] and skin secretions, and subsequent testing of the irradiated materials may reveal the presence of a carcinogen derived from a biologic precursor. The initiation of tumors by X-radiation and by radioactive materials may also involve the production of a chemical carcinogen, or, alternatively, may be a consequence of a direct effect on some cell structure or cell mechanism.

Even if chemical carcinogens should be found to be responsible for originating spontaneous tumors, this would throw light only upon what has been termed the "causal genesis" (*47*); the mechanisms of the "formal genesis" would still have to be elucidated.

## SUMMARY

Concluding the reports of the studies in this laboratory of the relationship between chemical structure of polycyclic compounds and the capacity to induce tumors at the site of injection, this paper gives data on 45 compounds injected into about 1,000 mice, chiefly strain A. Five of these

[6] *Preliminary experiments (M. J. Shear and J. W. Schereschewsky, unpublished work, 1936) gave negative results, but further exploration of this hypothesis appears warranted.*

compounds produced tumors at the site of injection.

A total of 181 compounds has been examined, and 60 of them have been found to exhibit carcinogenic potency.

The bearing of these findings on the problem of the genesis of spontaneous tumors is discussed.

Acknowledgment is made of the technical assistance of Adrien Perrault.

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# Quantitative Induction of Tumors in Mice With Ultraviolet Radiation

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So many investigators have produced neoplasms with ultraviolet radiation that there can be no question of the efficacy of this agent in carcinogenesis. Only Rusch, Kline, and Baumann (*1*), however, have made quantitative estimates of the incident radiation required. Their studies have provided a starting point for our attempt to quantitate further such measurements.

The method herein described has been developed to meet the need for reproducible quantitative measurements. Before discussing the details of the method, certain general problems confronting the control and measurement of radiation for such experiments need consideration.

## GENERAL CONSIDERATIONS WITH REGARD TO MEASUREMENT OF RADIATION

While it would be desirable to expose the animals to monochromatic radiation, this is not feasible because of the difficulty of obtaining monochromatic radiation of the required intensity over an area as large as a single mouse, let alone the numbers required for statistical treatment. (See p. 265.) Use of restricted spectral regions obtained by means of filters should be possible when certain basic information is available. (See p. 266.) For many purposes, however, sources of radiation which emit a high proportion of those wavelengths known to be active in cancer production may be satisfactorily employed. The action spectrum has been shown by Roffo (*2*), Funding, Henriques, and Rekling (*3*), and Rusch, Kline, and Baumann (*1*) to include only wavelengths shorter than about 3200A (cf. Blum (*4*) for a summary of the evidence [1]), and these are conveniently supplied by the mercury arc. Numerous investigators have suggested the participation of other wavelengths (or mechanisms which demand the participation of other wavelengths), but the above-mentioned studies show clearly that only those shorter than 3200A are obligatory, and that longer wavelengths probably do not affect carcinogenesis. From the experimental standpoint this is fortunate, since the sources available, among which the mercury arc is most satisfactory, all emit a considerable amount of energy outside the spectral range desired, and there is no satisfactory method for removing such radiation by means of filters.

For continuous measurement of radiation, a photocell is most satisfactory. Such an instrument is not uniformly sensitive throughout the spectrum. The titanium cell used in these studies is sensitive only to wavelengths shorter than 3150A which makes it particularly valuable for our purpose, since these are the wavelengths that induce tumors. The spectral sensitivity of this cell is shown in figure 1 together with the intensities of the various lines of a mercury arc. When this type of source is used, only the 3130A line and those of shorter wavelengths affect the photocell.

In estimating the intensity of the radia-

[1] *The paper of Funding et al. was not available at the time of publication of this review but has since been examined. Their data agree completely with the analysis there presented.*

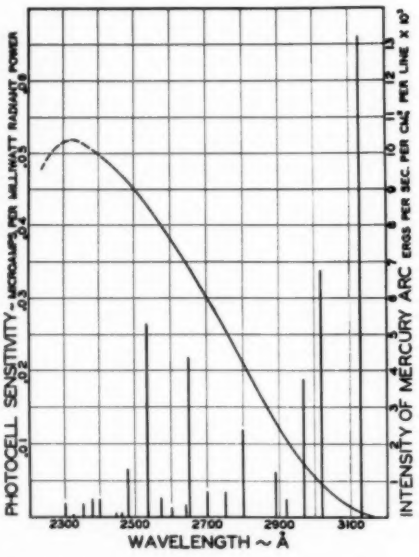


FIGURE 1.—Spectral sensitivity of photocell, spectral distribution, and intensities of lines of mercury arc.

tion it is necessary to weight each line according to the sensitivity of the cell for that particular line. Thus it is important that the intensity of the various lines with reference to each other does not change appreciably under the conditions of operation of the arc. The type of mercury arc used in these studies is particularly stable in this respect.

## PHYSICAL MEASUREMENTS

### General Assembly

The general assembly of the apparatus is shown in figure 2. The mercury arc, *L*, which serves as a source of ultraviolet radiation is mounted in a metal housing equipped with a semiparabolic aluminum reflector. The animals to be irradiated are placed upon a slowly revolving turntable at *T*. Continuous movement of the animals in the radiation field is necessary to insure uniform exposure, as a 20-percent variation in intensity over the area occupied by the animals is introduced due to the geometry of source and reflector. Since it is not feasible to operate the photocell used for controlling the dosage of radiant energy at the position of the animals, it is mounted above the lamp at a fixed position, *P*, where it receives a sample beam through an opening in the reflector and housing. The ratio of the intensity at *P* to the intensity at *T* is determined by calibration described below. The measured intensity at *P* multiplied by this ratio gives the intensity incident at *T*.

The entire apparatus is mounted in a metal cabinet equipped with forced ventilation in order to remove noxious gases produced by the lamp, and to prevent rise in temperature.

FIGURE 2.—Apparatus for exposing mice to mercury-arc radiation. The arc itself is hidden in the metal housing at *L*. The photocell is enclosed in the box at *P*. The turntable carrying the mice is at *T*.

### Light Source

The light source is a standard, commercial, therapeutic-type, intermediate-pressure [2] mercury arc in quartz (*5*). The relative intensities of the various lines are shown in figure 1. Spectra of this source measured [3] at intervals during the course of the experiments have shown that it is stable as regards the relative intensity of the lines. Total output fluctuates with line voltage, temperature, aging, etc.; but the only factor which could affect the dosage measurements is a change in relative intensity of the lines, and such has not occurred under our operating conditions.

### Photocell and Recorder

The instrument for radiant-energy measurement is an integrating photoelectric meter developed by Kuper, Brackett, and Eicher (*6*). The photosensitive element in this device is a photocell which is so arranged that when a given quantity of energy falls upon the photocell an electrical pulse activates a counting device. Thus each pulse recorded by the counter represents a given quantity of radiant energy. Energy is immediately expressed in terms of the number of pulses counted and intensity in terms of the rate of pulses. With such a device properly calibrated in ergs of radiant energy per pulse, the dosage and intensity [4] are at once available in physical units. Furthermore, since the instrument is of the integrating type, fluctuations and changes in the intensity of radiation incident on the animals due to line-voltage variations, temperature effects, solarization of the quartz envelope, and slow aging with diminution of over-all ultraviolet output of the arc are properly taken into account so far as dosage is concerned.

### Calibration of Photocell and Measurement of Intensity

The titanium photocell was initially calibrated against a standard photocell, which in turn had been standardized against a thermocouple. A large quartz double monochromator [5] was used in the calibration to insure monochromatic radiation of high purity. The spectral sensitivity is shown in figure 1.

For calibration in absolute units a previously calibrated low-pressure mercury arc was used. Such a source emits virtually no radiation to which the photocell is sensitive other than the 2537A line and hence may be considered a monochromatic source. By exposing the photocell to this source it was possible to determine the quantity of radiant energy of 2537A in ergs per cm.$^2$ per pulse of the photocell recorder.

However, since the photocell is not equally sensitive to all wavelengths, this calibration is only valid for 2537A. The energy per pulse when the photocell is used to measure the radiation emitted by the lamps employed in these experiments must be calculated from the relative intensities of the emitted lines and the sensitivity of the photocell to the corresponding wavelengths. The method is best explained by a sample calculation which is summarized in table 1. For simplicity only three lines are considered in the table, namely, 3020A, 3650A, and 2537A; the method is the same for the 20 lines that are

[2] *This term is used here to distinguish this type of arc, which operates at about atmospheric pressure of mercury vapor from arcs which operate at much higher or much lower pressure and whose emission spectra are quite different.*

[3] *For this purpose a large quartz double monochromator of recording type was used. This instrument was designed by Dr. F. S. Brackett and developed cooperatively by the Washington Biophysical Institute and the National Bureau of Standards. It will be described elsewhere in the near future.*

[4] *Intensity is energy per unit area per unit time, e. g., ergs/cm.$^2$/sec. Dosage is intensity $\times$ time, e. g., ergs/cm.$^2$.*

[5] *See footnote 3.*

TABLE 1.—*Method of calculating absolute energies recorded by the photocell* [1]

| Wavelength | Relative intensity of lines | Relative sensitivity of photocell | Relative response by photocell [2] | Percent of each count due to each wavelength | Absolute sensitivity of photocell and counter ergs/cm.²/count | Ergs/cm.²/count due to each wavelength [3] |
|---|---|---|---|---|---|---|
| 3020A | 1.5 | 2 | 3 | 15 | 1,200 | 180 |
| 2650A | 1.0 | 7 | 7 | 35 | 340 | 119 |
| 2537A | 1.25 | 8 | 10 | 50 | 300 | 150 |
| Total | | | 20 | | | 449 |

[1] The values are not actual, but assumed for purposes of illustration.
[2] Column 2 times column 3.
[3] Column 5 times column 6.

actually encountered. The product of the relative intensity of a given line (column 2) and the relative sensitivity of the photocell for that wavelength (column 3), both obtained from the initial calibration data, gives an index of the relative response of the photocell (column 4). The sum of the relative responses for all the lines is taken as 100 percent, and the percent of each pulse contributed by each wavelength is calculated (column 5). The values in column 6 are obtained by multiplying the absolute calibration value for 2537A by the sensitivity of the photocell for the given wavelength relative to its sensitivity at 2537A. These values represent the absolute sensitivity of the cell for the various wavelengths. When multiplied by the corresponding contributed percentage (column 5), the number of ergs per cm.² contributed per pulse by each wavelength is obtained (column 7). The sum of these values gives the total energy in ergs per cm.² represented by one pulse of the photocell recorder when exposed to this particular source.

In order to obtain the total incident intensity, the total of column 7 should be multiplied by the number of pulses recorded per second when the photocell is placed in the position of the animals. In principle, the latter measurement is quite simple. The photocell unit of the photoelectric meter is placed at the position of the animals, a reading taken of the number of pulses per unit time, and the cell is then placed in its fixed integrating position in the sample beam above the lamp and the number of pulses per unit time again determined. The ratio of the rate of pulses for the position of the animals to that for the operating position is thus obtained. It must be noted that the sample beam should originate from a large enough area of the source to insure that local changes in the surface of the quartz enclosure envelope do not affect this ratio.

In practice, several complicating factors are encountered. Due to the nonuniformity of the radiation field, the cell cannot be placed at any one position during the measurement but must move through the field on the revolving turntable in order that the average intensity to which the animals are exposed can be measured. Also, the cell and recorder are far too sensitive to permit direct readings at the position of the animals, and some method for reducing the incident intensity to only a few percent of its actual value is necessary. For this purpose a screen was made by drilling small holes in a piece of 1-mil brass foil. Its transmission was 1.8 percent; it showed no appreciable change in transmission with respect to angle until the angle of incidence exceeded 45°, a value not reached in the positions employed.

Another factor which must be consid-

ered is the angle of incidence of the beam on the photocell itself. Even with no screen the angular acceptance of the photocell is somewhat limited due to its geometry. However, with the present arrangement this factor is not important unless lamp and reflector are within 50 cm. of the position of the animals. This has occurred in none of our experiments.

### Variation of Intensity and Dosage Control

In a number of the present experiments it has been necessary to fix dosage at a constant value and to vary the intensity and duration of the exposure. Intensity control has been accomplished in most instances by the use of calibrated wire screens placed between the source and the animals; in a few experiments subsequent to those described in this paper, the intensity was also varied by changing the distance between lamp and animals.

In addition to these controlled variations, the slow aging of the lamp introduces a diminution of intensity which must be taken into consideration. Although the integrating counter keeps the dosage constant no matter how much the lamp falls off in intensity, it has been desirable to keep the basic intensities as fixed and constant as possible, and this has been accomplished in our later experiments by lowering the source as the lamp ages. Thus the incident intensity can be adjusted to its original value from time to time.

## INDUCTION OF TUMORS

### Animals

In order to avoid as many variables as possible, one sex of a genetically pure strain of mice was used. The mice were males of the albino strain A, supplied by the Roscoe B. Jackson Memorial Laboratory. They were approximately 6 weeks old when received and 2 to 3 months old when first irradiated.

The mice were fed Purina fox chow. They grew well, as a rule; but there was slower growth in irradiated animals, the inhibition of growth being greater with greater doses of radiation.

### Irradiation

Seven series of mice were irradiated in this first set of experiments, there being 26 animals in each series at the start. They were irradiated 5 times weekly—each day except Saturday and Sunday. A few isolated days were missed from this schedule, 9 in all, in the course of 1 year. Irradiation of series A, B, C, and D began in July, and of F, G, and H in August 1940.

The dosages and intensities of radiation were different for each series; they are listed in table 2. The dosages were determined as described above by the number of pulses from the photocell recorded by the integrating counter, the irradiation being terminated when the required number of pulses had been recorded. Thus the total dosage given is not affected by differences in intensity, which reflect differences in operating conditions of the lamp and which alter the rate of the pulses and hence the duration of the irradiation period. The relative dosages received by

Table 2.—*Incident intensities and dosages of radiation of wavelength 3130A and shorter*

| Series | Daily dose ergs/cm.²/day | Average intensity ergs/cm.²/sec. | Days to 50 percent tumors $t_{50}$ | Total dosage to $t_{50}$ ergs/cm.² |
|---|---|---|---|---|
| A | $8.61\times10^{7}$ | $4.3\times10^{4}$ | (166) | $(10.2\times10^{9})$. |
| B | $3.29\times10^{7}$ | $1.6\times10^{4}$ | 155 | $3.6\times10^{9}$. |
| F | $3.36\times10^{7}$ | $4.3\times10^{4}$ | 135 | $3.2\times10^{9}$. |
| C | $1.96\times10^{7}$ | $.97\times10^{4}$ | 143 | $2.0\times10^{9}$. |
| G | $1.98\times10^{7}$ | $4.3\times10^{4}$ | 147 | $2.1\times10^{9}$. |
| D | $.758\times10^{7}$ | $.37\times10^{4}$ | 324 | $1.76\times10^{9}$. |
| H | $.776\times10^{7}$ | $4.3\times10^{4}$ | 290 | $1.60\times10^{9}$. |

the various series of animals were accurately measured, even though the intensity varied considerably from time to time due to fluctuations of the arc. In the earlier part of this experiment the intensities, and hence the durations of the irradiation periods, varied as much as $\pm 25$ percent from the mean. The intensity was controlled within much narrower limits in the latter part of this and subsequent experiments. The data in table 2 indicate that no serious error was introduced by variations in intensity so long as the dosage was accurately controlled, and, moreover, all series were subjected to approximately the same variations. The average daily duration of the irradiation for the different series ranged from about 4 to 45 minutes.

The errors in measurement refer to energy incident in the approximate plane of the animals, as measured by the photocell. Actually the mice do not remain quiet, but change their positions within the limited confines of their individual cages, as is illustrated in figure 3. In the following incidence studies only tumors of the ear are considered since these areas are uniform, and being little protected by hair, they are the sites of the great majority of the tumors. The figure shows that the animals often take positions such that the ears are not exposed to the radiation, but we have no reason to believe that a given mouse habitually takes any given position since they move about frequently. The wires of the cages cut off a certain part of the incident radiation, about 20 percent, but the ears of the animals project between the meshes of the wire during a large portion of the time. It seems safe to assume that the various positions of the mice are distributed randomly in the course of the experiment, and thus tend to equalize any effects of shading either by the cages or the bodies of the animals themselves. Hence, the incident energies estimated by means of the photocell should provide a valid comparative index of the energies incident on the mice's ears but cannot be regarded as absolute values in this sense.

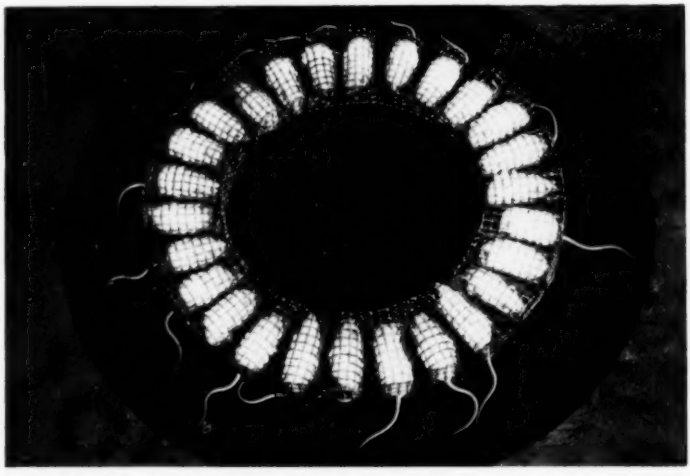

FIGURE 3.—Disk of turntable with mice in individual cages. Note differences in position of animals.

## Incidence of Tumors

Figure 4 shows the incidence data for our first seven series. Only tumors of the ears are considered. The criteria for their selection will be discussed, together with the histologic studies, in the following paper. In the figure, the logarithm of the number of days until appearance of the tumor is plotted against the percent of tumors which have appeared at that time. Plotted in this way, the data follow rather symmetrical S-shaped curves. Plotted as days against percentages, the curves are, of course, considerably skewed. There is a wide spread between appearance of the first and last tumors in each series. For example, in series F the first tumor appeared at 102 days, the last at 221, while the 50-percent incidence was reached at 135 days. This wide spread is particularly striking in a genetically pure strain and clearly illustrates the necessity of using relatively large numbers of animals for such experiments.

The percentage of tumors at any given time is calculated as follows:

$$\frac{T\times 100}{N-D}=\text{percent of tumors}$$

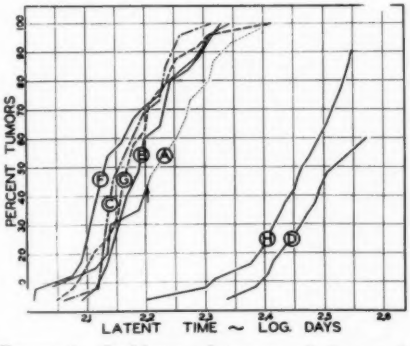


Figure 4.—Incidence of tumors of the ear in male strain A mice exposed to ultraviolet radiation. For dosages of radiation, see text. Irradiation of series A ceased at the time indicated by the vertical arrow.

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where $N$ is the total number of mice initially irradiated (usually 26), $T$ the number with tumor, and $D$ those that were killed or had died prior to that given time. This seems the proper basis for this calculation if it can be assumed that the animals comprising $D$ died or had been killed for causes not related to tumor development. It is obvious that high mortality may influence the shape of the curves, if this or any other method of calculating percentages is employed. On the whole, the mortality was so low as not to modify greatly the shape of the curves. In both series A and B, 2 mice were killed for histologic examination before the first tumor had appeared. There was no mortality, and no animals were discarded in series C. In series F, 1 animal was discarded because it was a relatively hairless type found rarely in this strain and was considered atypical. In series G, 1 animal died during the experiment, and 1 developed a tumor of the eye and was discarded, as has been our practice in those rare instances in which the tumor of the eye has made it necessary to kill the animal before a tumor of the ear appeared. In series D and H, the mortality up to the occurrence of 50 percent of tumors was not high, 11 and 8 percent, respectively, but increased considerably after this point, since the animals were approaching their average life span. This accounts, in all probability, for the divergence in the latter half of these curves. Series A, which received the highest dosage, showed considerable early mortality, and it was necessary to discontinue the irradiation before 50-percent tumor occurrence had been reached. However, all the animals that survived developed tumors. In this series the diet was supplemented during a part of the time by bread and milk, since the animals' snouts were severely irritated and they had difficulty in feeding on the dry

fox chow. This series, obviously, has little statistical significance, but it is of interest in establishing the highest limit of dosage which it is feasible to employ. In series B, F, C, and G, and the first half of D and H, the mortality was so low as not greatly to affect the shape of the curves.

While it is possible to obtain 100-percent tumor incidence by irradiation, the incidence of spontaneous tumors of the ear in mice of this strain is extremely rare. No such tumors appeared in control series comprising 75 animals maintained under like conditions but not irradiated. Among about 8,000 mice of this strain observed in this laboratory, only 5 apparently spontaneous tumors of the ear have been noted.

When plotted as logarithm of days against probability units, the data follow straight lines with a reasonable degree of scatter. More exact analysis of the data will not be attempted at this time but will be deferred until the results of further experiments now in progress are available.

### Dosage and the Reciprocity Law

Since the main object of these first experiments was to establish the dosage requirements, it was necessary to determine whether the intensity of the radiation influences tumor formation. That is, is the reciprocity law

$$\text{Intensity} \times \text{time} = \text{constant}$$

obeyed? Obviously, it would be difficult to determine this directly so long as we do not know to what extent the rate of tumor formation is dependent upon radiation.

Fortunately in the present instance the experimental conditions permit an indirect approach to the problem. Since the period of irradiation is a small fraction of the total day, it is possible to give the same daily dosage at a high intensity for a short time, or at a lower intensity prolonged over a correspondingly longer period, and to compare the results in the two cases. Thus, in series F, G, and H, the maximum intensity obtainable was used for short periods, whereas series B, C, and D, respectively, were subjected to corresponding doses but at lower intensities. If intensity is of no importance and dosage alone determines the occurrence of the tumor, the time to its appearance should be the same for series B and F, for series C and G, and for series D and H. That this is approximately true is shown in table 2 where total times and dosages required for occurrence of 50-percent tumors are shown, together with average intensities for each series. As may be readily seen, there is an approximate correlation between total dosage and time to occurrence of tumors, but there is no correlation between intensity and the latter value.

For each series pair receiving approximately the same dosage, (e. g., B and F) the difference in time to 50-percent tumor incidence does not exceed 12 percent. It is probable that these differences represent the variation between the individuals of the different series which are each composed of 26 animals. This is borne out by the fact that the variation between series B and F, in which the difference in intensity is about twofold, is as great as the variation between D and H, in which the intensities differ over tenfold. The extent of variation between identically treated series is being studied at present.

The data thus far obtained permit the conclusion that intensity per se is not an important factor, but that tumor development under a given schedule of irradiation is dependent only on total dosage. Experiments which further test this relationship are in progress.

By knowing that the reciprocity law holds, the setting up of other experiments is simplified. For example, in any attempt to study the effectiveness of different

wavelengths, it is not feasible to adjust the effective intensities of radiation of various wavelengths to a common value; but if the reciprocity law holds, this is unnecessary since only the effective dosage need be controlled. The latter is much the more easily accomplished.

Adherence to the reciprocity law suggests that the photochemical processes underlying tumor production are not complicated by exactly simultaneous reverse processes. However, this does not exclude the possibility that resultant products are continuously carried away, or that other "recovery" processes take place.

The data in table 2 also show that at high dosages a given increment in total dosage has much less effect on time to tumor occurrence than has a corresponding increment at low dosage. The data thus far obtained do not permit a more accurate analysis of the relationship between dosage and time to tumor appearance. They indicate, however, that in experiments designed to determine the effect of various agents, e. g., diet, on the development of tumors induced by ultraviolet radiation, the dosage must be carefully selected and controlled. If too high dosage is employed, the time to tumor formation should not be greatly affected by such modifying factors since it is little affected by total dosage under these conditions. If the dosage is too low, the time to tumor occurrence will be prolonged into the range of the average life span of the animals and vitiated by high mortality. Other factors of importance in this respect are being subjected to experimental study at present.

For numerous reasons, no exact comparison can be made between our experiments and those of Rusch, Kline, and Baumann (*1*), as regards the dosages of radiation required to produce tumors. Certainly there is no great discrepancy. Our range of intensities and dosages includes and extends that studied by those investigators.

Very little will be said at this time regarding the magnitude of the dosages of ultraviolet radiation employed in these studies, as compared with that incident in sunlight, to which man may be subjected. Calculated in terms of accepted values for human skin, series D and H received about 20 to 25 minimal erythema doses per day. This value is considerably higher than that for sunlight. However, this basis of comparison is not a fair one, as a number of factors would tend to make this quantity of mercury-arc radiation less effective than the corresponding quantity of sunlight. Adherence to the reciprocity law suggests that prolonged exposure to the relatively low intensities of sunlight may be effective if incident over sufficient time.

As regards the effects of such dosage in mice, it may be pointed out that the D and H dose produces only slight erythema of the ears when the animal is first subjected to it. The higher doses produce correspondingly more pronounced effects, those to which series A was subjected being highly destructive. The nature and extent of the skin changes produced by various dosages of ultraviolet radiation will be the subject of a later paper.

## SUMMARY

A method for exposure of mice to mercury-arc radiation under carefully controlled conditions is described.

One hundred-percent incidence of tumors of the ears of male strain A mice may be induced by such radiation.

At higher dosages of radiation the time to appearance of the tumor is little affected by the dosage, but at lower dosage this time increases markedly with decreasing dosage.

The production of tumors depends upon the quantity of radiant energy applied, rather than upon the intensity of the radiation.

ACKNOWLEDGMENTS: Selection of appropriate dosages of radiation was greatly facilitated by measurements with our photocell, of the intensities used by Doctors Rusch, Kline, and Baumann in their experiments, and we are deeply indebted to these investigators for permission to make these measurements in their laboratory. It is a pleasure to acknowledge their ready cooperation in this and other matters pertaining to the problem.

Thanks are also due Dr. F. S. Brackett and the Washington Biophysical Institute for apparatus made available to us, to M. Eicher for calibrating our photocells, and to A. W. Murray and L. W. Lindner for valuable assistance in carrying out the routine irradiations.

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# Pathology of Tumors of the External Ear in Mice Induced by Ultraviolet Radiation

By Hugh G. Grady, *acting assistant surgeon*, Harold F. Blum, *senior pharmacologist*, John S. Kirby-Smith, *assistant physicist*, *National Cancer Institute, National Institute of Health, United States Public Health Service*

This paper deals with the gross and histologic features of tumors of the external ear induced in male mice of strain A by ultraviolet radiation according to the technique discussed in the preceding paper (*1*). Details of dosage, intensity, and incidence data may be found therein. Tumors of the external ear have been adopted as the basis for incidence studies since in a total of 147 tumor-bearing mice the external ear has been involved in 144. Tumors of the eye have developed in 6 mice, 3 in combination with tumors of the ear and 3 without tumor elsewhere. These latter have not been included in the incidence data, and the 3 animals bearing tumors of both eye and ear have been listed as positive for statistical purposes only when the tumor of the ear appeared.

When a tumor appeared on the external ear the animal was killed by cervical dislocation, and both ears and cervical lymph nodes were immediately placed in Zenker's fluid containing 5 percent of acetic acid. Histologic examination of the ears and cervical nodes was made in every instance, and any other lesions observed at autopsy were also examined histologically. Tissues were embedded in paraffin. The haematoxylin-eosin stain was employed routinely; other stains employed on occasion were Mallory's phosphotungstic-acid haematoxylin, Mallory's aniline blue, and Wilder's or Foot's methods for demonstrating reticulum.

## GROSS PATHOLOGY

The animals were examined twice weekly during the course of treatment, and the gross diagnosis of tumor was made, with very few exceptions, by the same observer, so that the criteria for selection have been as uniform as the nature of the material permitted. Obviously the early diagnosis of tumor by inspection and palpation is somewhat arbitrary but in every case in which a clinical diagnosis was made, histologic examination confirmed the opinion. The tumors varied somewhat in size depending largely on the shape and location of the growth. For example, tumors occurring on the middle of the inner surface of the ear were for the most part round, elevated, and sharply circumscribed lesions approximately 7 x 5 x 3 mm. (fig. 1). In a few instances there was diffuse involvement of the entire margin of the auricle in cordlike fashion, the longest measurement ranging as high as 18 mm., but the diameter of such growths rarely exceeded 3 or 4 mm. Not infrequently multiple growths were found either on the same ear or involving both ears. In such cases the presence of a single definitely neoplastic nodule was considered significant for incidence studies, and the animal was then killed.

In most cases the external surface of the tumor was pinkish-red in color and often superficially ulcerated. The more extensively ulcerated growths were frequently capped by a dry hemorrhagic crust, but "weeping" or freely bleeding lesions were unusual. The tissue was usually firm, dry, of a homogeneous gray-white cast, and on close inspection the cartilage plate could be distinguished as a thin grayish-yellow line. Microscopically both sar-

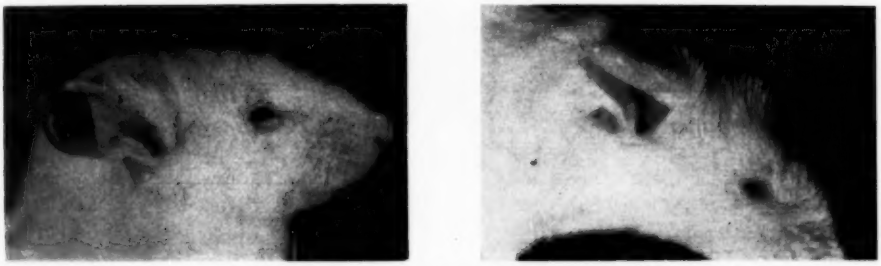

FIGURE 1.—*A*, Tumor involving inner surface of auricle; *B*, Tumor involving margin of auricle. Natural size.

comas and carcinomas occurred, but we were unable to distinguish with certainty between the gross appearance of sarcoma and carcinoma.

Associated nonneoplastic lesions in general varied with the total dosage of radiation. In the higher dosage groups, particularly in series A, the ears were thickened, deeply notched, and covered with grayish-yellow scales. Crusted excoriations were also frequent not only on the ears but also on the skin of the face, which was usually depilated as the result of the irradiation. The ears of animals in series D and H, which received low dosages, generally exhibited only minor degrees of scaling and thickening in nontumorous areas. With the exception of tumors of the eye, which will be described in another paper, the only other tumors observed were one fibrosarcoma of the forepaw and a few squamous papillomas of the tail.

## HISTOLOGIC OBSERVATIONS

The incidence of histologic types of tumors is given in table 1. It is seen that the tumors were sarcomas, carcinomas, or combinations of sarcoma and carcinoma. Combinations included independent carcinomas and sarcomas on the same or contralateral ear as well as so-called collision tumors in which the two types were intermingled. In each of series B, F, G, and H there was a single case in which the diagnosis of carcinoma was doubtful although the sarcomatous element was unquestioned. These are listed in parentheses in the respective columns.

### SARCOMAS

Histologically the sarcomas were quite uniform in type. They consisted almost exclusively of intertwining bundles of elongated spindle-shaped cells with moderately large, plump, and darkly staining nuclei (pl. 1, *A*). The cytoplasm was generally abundant, basophilic, and frequently finely fibrillar. With phosphotungstic acid-haematoxylin preparations, typical delicate blue fibroglia fibrils could be demonstrated in most instances. The reticulum in silvered preparations was abundant and consisted of a close meshwork of delicate strands closely applied to individual cells (pl. 1, *B*). Relatively small amounts of collagen were observed. Mitotic figures were numerous in practically all cases. Not uncommonly, large

TABLE 1.—*Incidence of histologic types of tumor*

| Type of tumor | Series | | | | | | |
|---|---|---|---|---|---|---|---|
| | A | B | C | D | F | G | H |
| Sarcoma | 10 | 20 | 22 | 7 | 20 | 19 | 14 |
| Carcinoma | | 1 | 1 | | 1 | | |
| Sarcoma and carcinoma | 5 | 3 (1?) | 3 | 4 | 3 (1?) | 4 (1?) | 3 (1?) |

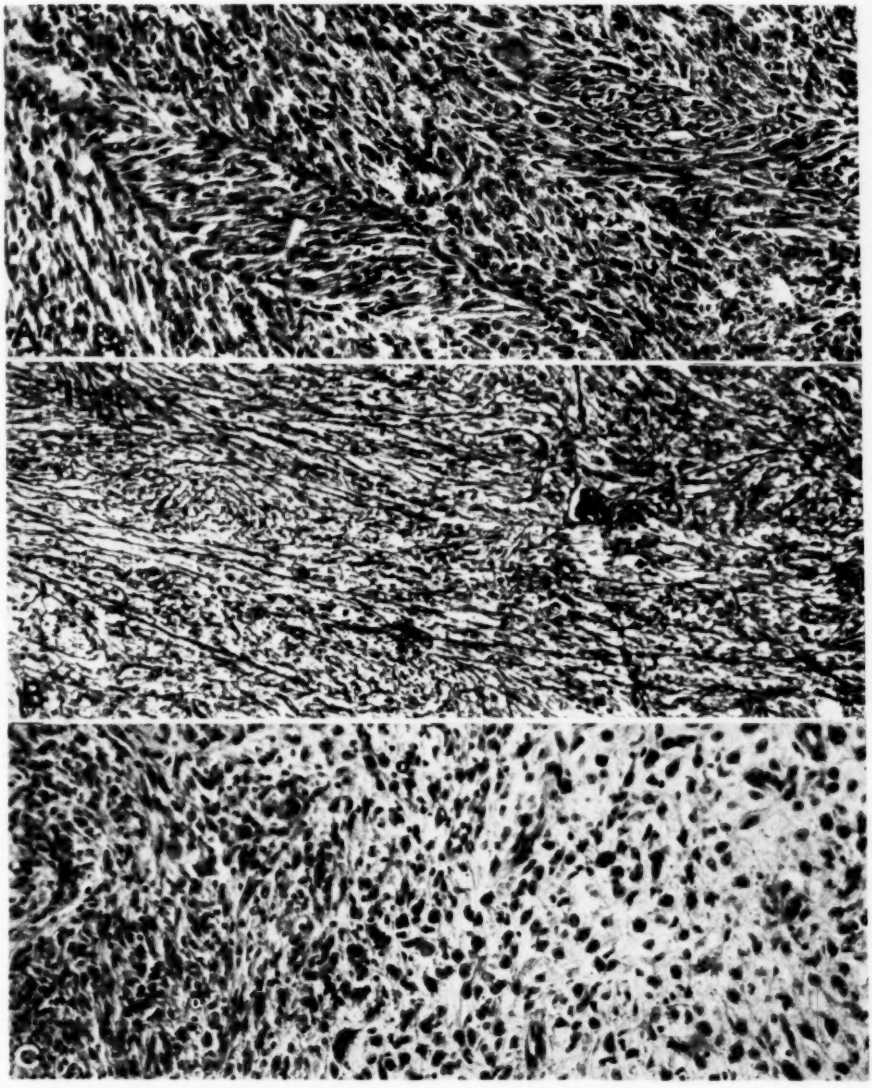

PLATE 1.—*A*, Fibrosarcoma composed of interweaving bundles of spindle cells. Phosphotungstic acid-haematoxylin. ×200; *B*, Reticulum pattern in fibrosarcoma. Foot's method. ×135; *C*, Edematous area in fibrosarcoma adjoining more typical area. Haematoxylin and eosin. ×180.

single or multinucleated giant tumor cells were seen. Blood vessels for the most part were inconspicuous, thin-walled, tortuous structures.

The greater bulk of such tumors was usually on one or the other side of the cartilage plate, although frequently strands of tumor cells had grown through a hiatus

in the cartilage and infiltrated the tissues on the other side in varying degree. Many sarcomas extended superficially to abut directly on the epidermis, which occasionally appeared atrophied or even ulcerated, and the sarcoma reached the free surface. In other areas, hyperplastic epidermis frequently dipped deeply into the sarcoma in long fingerlike projections, but these structures could usually be distinguished from truly carcinomatous foci by the integrity of the basal layer, the orderly stratification of the component cells, and continuity with the epidermal surface.

In a few cases large or small areas of the tumor were composed of polymorphous spindle-shaped, oval, round, or star-shaped cells widely separated by a clear, structureless, intercellular material (pl. 1, *C*). The close relationship with typical areas of fibrosarcoma and the presence in occasional cells of fibroglia lead us to believe that they represent edematous foci of fundamentally similar cytologic type.

### Carcinomas

The carcinomas, pure or combined with sarcoma, were all of the squamous-cell type and with few minor exceptions showed but little variation. On a basis of comparison with the grading of cutaneous carcinoma in man they ranged from type I to type III (pl. 2). They were almost invariably ulcerated growths which invaded the underlying tissue to the level of the cartilage and occasionally broke through the cartilage plate. There were broad masses, small islands, or strands of darkly staining polygonal cells, frequently slightly separated from each other, and with occasional masses of keratin or well-formed "pearls" in the center of such accumulations (pl. 2, *B*). The nuclei were large, and mitotic figures averaged one or two per high-power field. In one case in which a squamous carcinoma was intermingled with a fibrosarcoma, the carcinoma cells consisted of slender strands of closely packed cells with well-marked keratinization of the more central cells individually or in groups, but without typical pearl formation. The stroma in pure squamous carcinomas was usually scanty except about the advancing edges of the tumor where an active granulation tissue was present. However, this did not differ essentially from the granulation tissue of the ear resulting from the radiation in cases in which no tumor was present. The reticulum pattern in carcinomas was strikingly different from that occurring in sarcomas. The carcinomatous reticulum was relatively sparse and delimited large groups of tumor cells rather than individual cells (pl. 2, *C*).

In three instances collision tumors were observed, i. e., areas of squamous carcinoma intimately intermingled with, yet easily distinguishable from, sarcoma. In these, the sarcomas were large growths involving the greater part of the ears, while the epithelial tumors were smaller and generally more superficial. The disparity in size suggests that the sarcomas arise earlier or grow more rapidly than do the carcinomas.

### Metastasis

Metastasis was observed only once, in an animal in group H which had been irradiated for 283 days. There was a firm, reddish-gray tumor 8 x 5 x 5 mm. on the posterior margin of the left ear, and the left superior cervical nodes were slightly enlarged. Histologic sections of the primary tumor of the ear revealed a fibrosarcoma with invasion of lymphatic vessels. The enlarged cervical lymph nodes were partially replaced by a tumor of similar pattern which in one area had broken through the capsule and invaded the perinodal tissue (fig. 2).

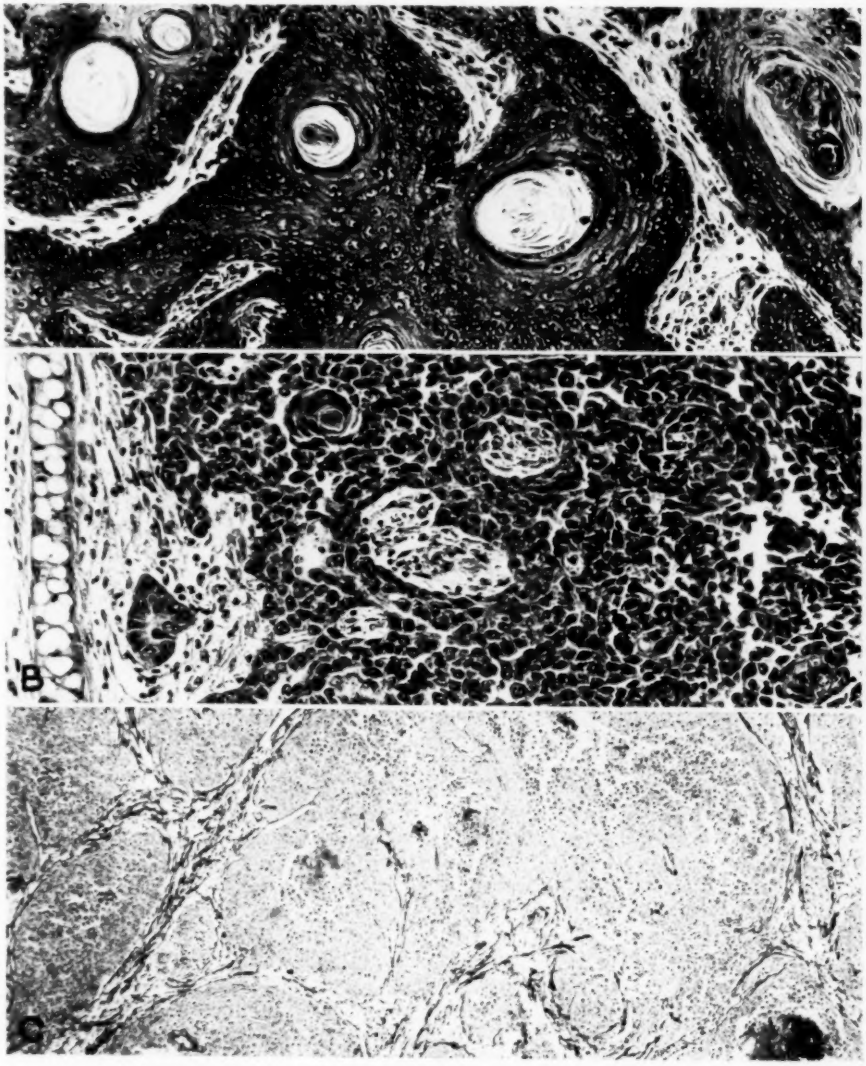


PLATE 2.—*A*, Well-differentiated squamous carcinoma. Haematoxylin and eosin. ×210; *B*, Squamous carcinom showing cellular separation and little keratinization. Haematoxylin and eosin. ×210; *C*, Reticulum pattern in squamous carcinoma. Compare with plate 1, *B*. Foot's method. ×135.

## DISCUSSION

The preponderance of sarcomas in these experiments is striking particularly in view of the results of previous investigators. Findlay (*2*) described cutaneous papillomas and carcinomas in mice following ultraviolet irradiation. Some of the carcinomas were frankly squamous in

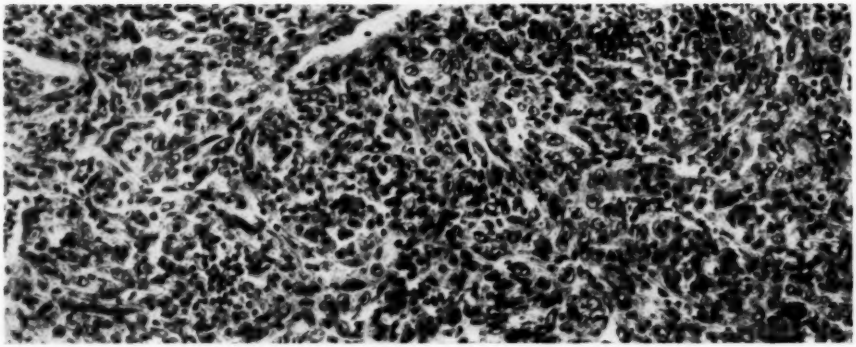

FIGURE 2.—Metastatic fibrosarcoma in cervical lymph node. Phosphotungstic acid-haematoxylin. ×220.

type, but there were spindle-cell growths including one which Findlay believed he could trace to epidermal cells. He raised the question "as to whether this tumor is to be regarded as a carcinoma or a carcinosarcoma."

Herlitz, Jundell, and Wahlgren (*3*) produced tumors on the external ears and tails of white mice. Many of these tumors were squamous-cell carcinomas; in addition there were spindle-cell growths which bore some resemblance to sarcoma but which, in the opinion of the authors, were epithelial in origin. This view seems to be based on the occurrence of transition forms between undoubted epithelial tumor cells and spindle-shaped tumor cells. Putschar and Holtz (*4*), working with rats, state in their first paper that squamous carcinoma was the most frequent histologic type encountered, with smaller numbers of basal-cell carcinomas and spindle-cell tumors which are regarded by some as "carcinosarcoma" but which these authors regard as an immature type of carcinoma. Certainly the tumor illustrated in figure 14 of their report while containing many elongated cells lacks many features of unequivocal sarcoma. In a later communication, which does not specify whether the observation was made on additional material, Putschar (*5*) reports the production of a collagen-producing sarcoma as well as a chondrosarcoma.

In Roffo's series (*6*) of 165 rats which developed tumors 145 had tumors of the external ear of which 58 percent were carcinomas, 36 percent sarcomas, and 6 percent carcinosarcomas.

Beard, Bogess, and Von Haam (*7*) induced tumors in 12 rats including 9 carcinomas of the external ear, 6 sarcomas of the eye, and 1 carcinoma on the skin of the nose. They make no mention of sarcomas involving the ears, and their figures are those of squamous carcinoma of varying histologic appearance.

Hueper (*8*) irradiated congenitally hairless rats and their haired litter mates. Only one of the hairless rats developed cutaneous carcinoma while multiple carcinomas and sarcomas were produced in the haired animals. He believed that the difference in incidence of tumors was due to the thickness and keratinization of the hairless skin which "provides a higher degree of protection against the ultraviolet rays."

In none of these communications, with the exception of Putschar's second note, is there any mention of collagen, reticulum, or fibroglia in those spindle-cell

growths which were believed to represent atypical carcinomas.

In our material the tumors diagnosed as sarcoma in most instances exhibited the characteristics of fibrosarcoma with fibroglia fibrils, and the reticulum was of a pattern typical of fibrosarcoma (*9*), being fundamentally different from that observed in squamous carcinomas. The amount of collagen as shown by Mallory's aniline blue or phosphotungstic acid-haematoxylin methods varied considerably. The predominant pattern of interweaving bundles of spindle-shaped cells in contrast with the sheets or islands of elongated cells seen in spindle-cell epidermoid carcinoma seems further evidence that these tumors are truly of connective-tissue origin.

The occurrence of but a single metastasis among 144 mice with tumors of the external ear seems due in part at least to the fact that the mice were killed as soon as a clinical diagnosis of tumor was made. However, it should be pointed out that most investigators have found relatively few metastatic tumors even in animals which had lived for longer periods. The greatest proportion is reported by Wahlgren (*10*) who found 14 animals with metastatic tumors in 2 series totaling 29 mice and including pulmonary and splenic metastases.

The bearing of studies such as the present on the importance of sunlight as an exciting agent in human cutaneous cancer is of particular interest. The difference in the distribution of tumor types in the two cases is most striking, sarcomatous growths predominating in the case of mice, whereas the vast majority of malignant tumors of human skin are carcinomas. This discrepancy may be explained in part at least by the difference in penetration of the tumor-producing radiation (wavelengths shorter than 3200A) into the skin in the two cases. Direct measurements in this laboratory show that human epidermis absorbs virtually all such radiation, so that its direct effects are limited to the epidermis, and hence it would be expected to produce only carcinomas. On the other hand, a great part of such radiation penetrates to the dermis of the mouse, so that it is not surprising that sarcomas result. The epidermis, of course, also absorbs a certain proportion of the radiation, so that carcinomas as well as sarcomas are to be expected.

Thus, to account for the high proportion of sarcomas in proportion to carcinomas, it may be necessary to assume that the connective tissue of the dermis is more susceptible to the production of tumors by this agent, or, alternatively, that the sarcomas develop earlier than carcinomas. If the latter is true, the early selection of the tumors practiced in the present studies would favor the finding of a higher proportion of sarcomas.

## SUMMARY

The pathologic features of tumors of the external ear induced in male strain A mice by ultraviolet radiation are presented. The predominant type of tumor observed was a fibrosarcoma.

Less frequently combinations of fibrosarcoma and squamous carcinoma occurred, while only three instances of squamous carcinoma occurred alone.

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# The Comparative Proteinase and Peptidase Activities of Rat Hepatoma and Normal and Regenerating Rat Liver

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## INTRODUCTION

Attempts have been made in recent years to compare the proteolytic systems of rapidly growing neoplasms with those of various normal tissues. Until now, suitable normal control tissues have not been available. The present report deals with the proteinase and peptidase activities of a hepatoma of the rat and of the normal and the regenerating rat liver. This set of tissues is particularly suitable for comparison because both the hepatoma and the regenerating liver exhibit rapid tissue proliferation in contrast with the normal liver. It is reasonable to assume that synthesis of specific cellular proteins takes place much faster in the two rapidly growing tissues than in the normal liver. Since enzyme concentration is probably a factor in the synthetic activities of these enzymes, an attempt was made to correlate their lytic activities in a general way with the growth rates of the respective tissues. Therefore, experimental conditions were chosen which gave relative concentrations and activities of these enzymes under optimal conditions for lysis.

After continued feeding of *p*-dimethylaminoazobenzene to albino rats, neoplasms arose in the liver which grew when transplanted subcutaneously. A study of the histologic changes which occurred after transplantation suggested that the tumor was derived from parenchymal cells of the liver (*1*).

Partial hepatectomy in the albino rat accelerates the growth rate of the residual liver as indicated by increase in mass and number of cells. This phenomenon, commonly called regeneration, is essentially a restoration or re-creation of liver tissue by proliferation of hepatic cells. Higgins and Anderson (*2*) and Brues, Drury, and Brues (*3*) have shown that increase in mass of the liver is greatest during the first 3 days after partial hepatectomy. The increase in cell number is most marked during the second and third days after the operation. A second period of accelerated weight increment also occurs, but this is not associated with any marked increase in the rate of cellular proliferation (*3*).

## EXPERIMENTAL METHODS

### PREPARATION OF TISSUE EXTRACTS

The hepatoma tissue was obtained from tumors grown in subcutaneous transplants of hepatoma No. 31 which was originally produced by Dr. Julius White, of this laboratory, by feeding *p*-dimethylaminoazobenzene to Osborne-Mendel strain rats for 7 months (*1*). The viable tissue in these transplants was separated from the necrotic material as completely as possible and was used immediately.

The regenerating liver tissue was obtained from 100-gm. Osborne-Mendel strain rats after excision of the median and lateral lobes of the liver under ether anesthesia. The excised portion represented about two-thirds of the total weight of the liver. Three days after operation the weight of the liver was approximately 85 percent of the normal weight as calcu-

lated on the basis of a liver-body weight ratio of 6 percent.

All of the rats were killed by decapitation and thoroughly drained of blood. The livers of the partially hepatectomized rats as well as the control livers of the normal 100-gm. rats were perfused with Ringer's solution. The livers and the hepatoma tissues were ground and extracted immediately with 4 volumes of acetate buffer at pH 3.5. This original extract was filtered through a fine mesh gauze and was used as the 1 : 4 dilution. It was diluted further with the pH 3.5 acetate buffer to give the 1 : 8, 1 : 16, and 1 : 32 dilutions. The total nitrogen per cubic centimeter in each of the diluted extracts was determined by digestion and direct nesslerization as described by Koch and McMeekin (*4*).

### The Proteinase Determinations

The proteinase activities of the three tissue extracts were tested by their ability to digest horse hemoglobin. The procedure followed was that of Anson (*5*, *6*) in which the degree of hydrolysis was measured by the amount of tyrosine color liberated during 10 minutes digestion by 1 cc. of enzyme (tissue) extract acting on 5 cc. of a 2.5-percent solution of hemoglobin at 38° C. The tyrosine color value was measured in milligrams of tyrosine, determined colorimetrically with the Folin-Ciocalteu reagent by using an Aminco type F electrophotometer. In those experiments in which cysteine HCl in a final concentration of 0.01 M was used to activate the enzymes, it was necessary to apply a correction for the decrease in color caused by the addition of formaldehyde as described in a previous report (*7*).

### The Peptidase Determinations

The peptidase preparations were made from the same tissues, hepatoma and normal and regenerating liver, by using a modification (*8*) of the method of Berger and Johnson (*9*) for leucylpeptidase. Since this method of preparation specifically concentrates the leucylpeptidase, the results obtained when *dl*-glutamylglycine was used as substrate may not have represented the maximum hydrolytic activities of the tissues. The digests were made by adding 1 cc. of the peptidase preparation to 3 cc. of pH 8.0 phosphate buffer, and they contained 0.05 millimoles of dipeptide per cubic centimeter. The degree of hydrolysis was determined by alkalimetric titration in alcohol according to the method of Grassmann and Heyde (*10*). The titrations were made after 2 hours and then daily for 4 days. The incubation temperature was 38° C. The total nitrogen content of these peptidase preparations (0.45 to 0.6 mg. N per cubic centimeter) could not be correlated with the peptidase activity.

### The Chemical Analyses of the Tissues

The original tyrosine color values of the extracts which were obtained as zero-hour determinations for the proteinase activities represented the sum of the tyrosine, tryptophane, and other phenolic split-products which were present in the extracts. The total nitrogen determinations showed the amount of nitrogenous material which was extracted from each tissue with the acid buffer.

The percent of total solids in the hepatomas and the perfused livers was determined by weighing before and after lyophilization. These lyophilized tissues were dried to constant weight and analyzed for nitrogen by the Koch and McMeekin method and for total sulfur by the Parr bomb technique.

## RESULTS

The proteinase activity of the hepatoma extracts was considerably greater than that of the extracts of normal and of regenerating rat livers (tables 1 and 2). Since the hepatoma extracts contained less nitrogen per cubic centimeter (table 3), this difference in activity became more marked when the degree of hydrolysis was based upon the nitrogen content of the extracts. Also, all of the hepatoma extracts tested in the 1 : 32 dilution showed proteinase activity while in some instances the normal and regenerating liver extracts showed no activity at this dilution. The extracts of the normal liver in the 1 : 32 dilution were active in three experiments without cysteine and in four experiments with cysteine. The extracts of the 24-hour regenerating liver in the 1 : 16 dilution showed activity in three of four experiments. With the 48-hour regenerating liver extracts, proteinase activity was demonstrated in two of four extracts in the 1 : 32 dilution, while one extract in the 1 : 16 dilution was inactive without the addition of cysteine. The two extracts of the 72-hour regenerating livers were both active in the 1 : 32 dilution, while only one of two extracts of 96-hour regenerating livers was active in this dilution. The three extracts of 7-day regenerating livers were active in the 1 : 32 dilution. These results indicated that not only the activity but also the quantity of proteinase extracted from the hepatoma tissue exceeded that from normal or regenerating liver tissues. Further evidence of the increased proteinase activity of the hepatoma extracts was seen in the higher percentage of active proteinase present since the addition of cysteine to the hepatoma digests caused only 40-percent increase in activity, whereas this increase with the normal and regenerating liver extracts varied from 63 to 100 percent.

TABLE 1.—*The proteinase activities*[1] *of extracts of rat hepatoma and of normal rat liver*

| Tissue extract | Dilution | Increase in tyrosine color value | |
|---|---|---|---|
| | | No cysteine | With cysteine |
| | | *Milligrams* | *Milligrams* |
| Rat hepatoma | 1:4 | 0.084 | 0.116 |
| | 1:8 | .079 | .128 |
| | 1:16 | .106 | .134 |
| | 1:32 | .071 | .100 |
| Average | | .085 | .119 |
| Normal rat liver | 1:4 | .043 | .057 |
| | 1:8 | .043 | .064 |
| | 1:16 | .039 | .072 |
| | 1:32 | .029 | .064 |
| Average | | .038 | .064 |

[1] The activities are given in increases in the tyrosine color values per milligram of nitrogen in the tissue extracts. The average values for four experiments with each tissue extract are given, except in the 1:32 dilution of the normal liver where 1 of the 4 extracts was not active without cysteine and the average values of 3 experiments are given.

TABLE 2.—*The proteinase activities*[1] *of extracts of regenerating rat liver and of a rat liver 24 hours after a mock operation*

| Interval after partial hepatectomy | Dilution | Increase in tyrosine color value | |
|---|---|---|---|
| | | No cysteine | With cysteine |
| | | *Milligrams* | *Milligrams* |
| 24 hours | 1:4 | 0.028 | 0.039 |
| | 1:8 | .034 | .047 |
| | 1:16 | .024 | .039 |
| Average | | .029 | .042 |
| 48 hours | 1:4 | .020 | .039 |
| | 1:8 | .056 | .066 |
| | 1:16 | .023 | .076 |
| | 1:32 | [2].021 | [2].075 |
| Average | | .030 | .064 |
| 72 hours [3] | 1:4 | .017 | .050 |
| | 1:8 | .034 | .044 |
| | 1:16 | .021 | .074 |
| | 1:32 | .039 | .057 |
| Average | | .028 | .056 |
| 96 hours | 1:4 | .031 | .043 |
| | 1:8 | .052 | .089 |
| | 1:16 | .037 | .076 |
| Average | | .040 | .069 |
| 7 days [4] | 1:4 | .037 | .056 |
| | 1:8 | .047 | .059 |
| | 1:16 | .048 | .097 |
| | 1:32 | .060 | .102 |
| Average | | .048 | .078 |
| 24 hours after mock operation | 1:4 | .044 | .064 |
| | 1:8 | .051 | .080 |
| | 1:16 | .036 | .094 |
| Average | | .044 | .079 |

[1] The activities are given in average increases in tyrosine color values for 1 mg. of nitrogen in the tissue extracts.
[2] Average of 2 experiments.
[3] Average of 2 experiments in all dilutions.
[4] Average of 3 experiments in all dilutions.

TABLE 3.—*The total nitrogen and the initial tyrosine color values* [1] *of extracts of hepatomas and of normal and regenerating rat livers*

| Tissue extract | Total N | Tyrosine color value | Tyrosine color value/N ratio |
|---|---|---|---|
| | *Milligrams* | *Milligrams* | |
| Rat hepatoma | 2.58 | 0.164 | 0.064 |
| Normal rat liver | 4.00 | .323 | .081 |
| Regenerating rat livers, interval after partial hepatectomy: | | | |
| 24 hours | 3.81 | .432 | .113 |
| 48 hours | 3.77 | .415 | .110 |
| 72 hours | 3.24 | .266 | .082 |
| 96 hours | 3.81 | .386 | .101 |
| 7 days | 3.73 | .398 | .107 |
| Liver 24 hours after mock operation | 4.0 | .257 | .064 |

[1] Average values from 3 experiments for 1 cc. of buffer extract in the 1:4 dilution are given.

The proteinase activities of the extracts of the regenerating livers for the first 3 days after partial hepatectomy appeared to be slightly less than that of the normal liver-tissue extracts which were tested. This difference in activity could not be attributed to the effects of surgical shock or ether anesthesia since the activity of the liver extracts obtained 24 hours after a mock operation was slightly higher than that of the normal liver extracts (table 2). In the later intervals (of 4 and 7 days) after the partial hepatectomy the proteinase activities of the regenerating liver extracts were slightly higher than those of the normal livers.

Although the 1:4 dilutions of the regenerating liver tissues contained about the same amount of total nitrogen as the same dilutions of the normal livers, the proportion of tyrosine and tryptophane, as indicated by the tyrosine color value, was higher (table 3).

The hydrolysis of *dl*-leucylglycine by the peptidase preparations from the rat hepatoma tissue was much more rapid and extensive than by the peptidase preparations from normal and regenerating livers (table 4). With three of four hepatoma preparations, all of the natural peptide of the racemic mixture (50 percent) was hydrolyzed in 2 hours, whereas this degree of hydrolysis was not attained by the normal or regenerating liver peptidases even after 5 days' digestion. In contrast with the very active hepatoma peptidase, two of four peptidase preparations from 24-hour regenerating liver did not hydrolyze *dl*-leucylglycine at all.

TABLE 4.—*The hydrolysis of* dl-*leucylglycine and* dl-*glutamylglycine by purified peptidases from rat hepatoma and from normal and regenerating rat liver, and from rat liver 24 hours after a mock operation*

[Average maximum hydrolyses obtained during 4 days digestion of the racemic peptide are given. 50 percent hydrolysis indicates that all of the natural peptide has been split]

| Source of peptidase | Hydrolysis | |
|---|---|---|
| | *dl*-Leucyl-glycine | *dl*-Glutamyl glycine |
| | *Percent* | *Percent* |
| Rat hepatoma | [1] 52 | 34 |
| Normal rat liver | 22.5 | 40 |
| Regenerating rat liver, interval after partial hepatectomy: | | |
| 24 hours | 8.2 | 21.7 |
| 48 hours | 32 | 35 |
| 72 hours | 28 | 35 |
| 96 hours | 32 | 39 |
| 7 days | 20 | 29 |
| Rat liver 24 hours after mock operation | 15 | 22 |

[1] This degree of hydrolysis was obtained in 2 hours.

There was no significant difference between the ability of the hepatoma and normal liver peptidases to hydrolyze *dl*-glutamylglycine. The lower percentage hydrolysis obtained with the 24-hour regenerating liver and the liver after a mock operation may have been due to the effects of the operation alone. At longer intervals after the partial hepatectomies the peptidases from the regenerating livers became more active.

The hepatoma tissue contained from 10 to 12 percent more water than the normal or regenerating liver tissues (table 5). There were only slight differences in the total nitrogen and total sulfur contents of the three dried tissues. Although the nitrogen content of the hepatomas ap-

peared to be slightly higher, the nitrogen: sulfur ratio of this tissue was approximately the same as that of the normal and the regenerating liver tissues.

TABLE 5.—*The concentrations of total solids, total nitrogen, and total sulfur in the hepatomas and in the normal and regenerating rat livers*

| Tissue | Total solids | Total N | Total S | Ratio N/S |
|---|---|---|---|---|
| Hepatoma: | *Percent* | *Percent* | *Percent* | |
| No. 1 | 19.52 | 13.15 | 1.05 | 12.52 |
| No. 2 | 17.50 | 12.88 | 1.12 | 11.50 |
| No. 3 | 17.09 | 12.66 | .98 | 12.92 |
| Normal liver: | | | | |
| No. 1 | 29.63 | 11.50 | .93 | 12.37 |
| No. 2 | 29.18 | 11.83 | 1.00 | 11.83 |
| No. 3 | 30.52 | 9.90 | .93 | 10.65 |
| Regenerating livers, interval after partial hepatectomy: | | | | |
| 24 hours | 29.85 | 10.80 | .89 | 12.13 |
| 48 hours | 29.63 | 11.50 | .89 | 12.92 |
| 72 hours | 28.50 | 11.70 | .89 | 13.15 |
| 96 hours | 29.80 | 10.10 | .89 | 11.35 |
| 7 days | 28.55 | 11.75 | .93 | 12.63 |

## DISCUSSION

A comparative study was made of the proteinase and peptidase activities of two tissues, hepatoma and regenerating liver, which were derived from hepatic cells. In both of these tissues the growth rate, with an accompanying synthesis of protein, was definitely increased over that of the normal hepatic tissue.

With the hepatoma extracts there was a direct correlation between increased growth rate and the increased proteolytic activity over that of the normal liver tissue. As suggested previously (*7*), the increased rate of synthesis in a neoplastic tissue may be due to changes in metabolism which provide a more stimulating tissue medium for enzymic synthesis, or to quantitative or qualitative changes in the cathepsin which may occur when a normal cell becomes neoplastic. The results of the experiments reported here, in which the enzyme was removed from its natural tissue medium, indicated that there was a quantitative increase in the cathepsin (tissue proteinase) content of the hepatoma extracts. There may also have been a qualitative change which caused the increased lysis of hemoglobin even in the most dilute extracts. The higher percentage of active enzyme in these extracts may have been due to a more reducing tissue medium of the hepatomas which preserved the enzyme.

The regenerating liver, which apparently proliferated more rapidly than the hepatoma, however, had less proteinase during the period of most rapid regeneration as judged by the lytic activity with the techniques employed. In our earlier research we showed that the concentration of substrate, pH, and oxygenation were factors which controlled the synthesis of protein in tissue extracts (*11*, *12*). It might, therefore, be possible to explain the rapid synthesis of protein in the regenerating liver *in vivo* partly on the basis of the nutrient quality of the portal blood. The weaker lytic activity of the proteinase of these regenerating liver extracts in the presence of adequate protein substrate and under optimal hydrolytic conditions is difficult to explain. It may be that further discussion of the role of the proteinase in regenerating liver must await knowledge of the specific stimulating and self-limiting factors in the growth of regenerating tissues.

There was also a decided acceleration of the leucylpeptidase activity of the hepatoma tissues over that of the normal or regenerating rat livers. Very little, if anything, is known concerning the metabolic relationships of the peptidases which have an alkaline pH optimum to the proteinase and peptidase activities of the cathepsins. This increased leucylpeptidase activity of the neoplastic tissue, however, suggests that another change in the protein metabolism of the hepatomas occurred.

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## SUMMARY

The proteinase activity of rat hepatoma extracts using hemoglobin as substrate was greater than that of normal or of regenerating rat-liver extracts. Also, *dl*-leucylglycine was hydrolyzed much more rapidly and extensively by the peptidase preparations from hepatoma tissue than by the peptidase preparations from the normal or regenerating hepatic tissues. This definite acceleration in both the proteinase and the leucylpeptidase activity suggests that the nitrogen metabolism of the hepatic tissue was altered when it became neoplastic, and this change in the nitrogen metabolism favored a more rapid growth rate in the tumor.

During the first 3 days after partial hepatectomy, which included the period of most rapid proliferation, the average proteinase activities of the regenerating livers appeared to be slightly less than those of the normal liver extracts. On the fourth and seventh day after the operation the average proteinase activities were slightly above those of normal hepatic tissue.

The hepatoma tissue contained from 10 to 12 percent more water than the normal or regenerating liver tissue. The total nitrogen content of the dried hepatoma tissues was very slightly higher than that of the normal or regenerating livers, although the nitrogen : sulfur ratios remained the same in all of the tissues.


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# The Liver Catalase Activity of Tumor-Bearing Rats and the Effect of Extirpation of the Tumors

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## INTRODUCTION

Direct chemical studies on tumors are interesting and important. Of equal interest are chemical studies of the properties of tissues in the tumor-bearing animal relatively far removed from the site of the tumor. The latter type of investigation may offer some evidence of possible systemic effects of far-reaching importance which are elicited by the presence of the tumor. The present investigation forms part of a general plan embracing on one hand comparative chemical studies of tumor and normal control tissues and on the other hand similar comparative studies of a particular bodily tissue from normal and from tumor-bearing animals. For the time being, attention is confined largely to the liver, with some brief attention to the spleen and kidney.

In a previous paper (*1*) it was shown that the catalase activity of the livers of rats bearing the transplanted hepatic tumor No. 31 (*2*) was invariably about one-tenth that of the livers of normal rats. The activities of several enzymes were investigated in this comparative study, but the most striking alteration was found in the catalase activity of the livers of the tumor-bearing rats. It was further shown that a proportionality existed between the ratios of the enzymatic activity of normal liver to the transplanted hepatic tumor on the one hand and that of normal liver to the livers of tumor-bearing animals on the other. The catalase activity of regenerating rat liver in otherwise normal animals was found to be practically identical with that of normal liver.

These observations raised several interesting questions, and it was considered desirable to extend further the study of the catalase activity of tumor-bearing animals. These questions are as follows:

(1) At what stage of growth of the transplanted tumor does the effect on the liver catalase begin to show?

(2) What is the effect of extirpation of the transplanted tumor on the activity of the liver catalase of the animal which has borne the tumor?

(3) Will a second inoculation of the tumor grow in animals from which the first transplant has been removed, and if so, what is the catalase activity of the livers of such animals during the growth of the second transplant and after extirpation of the latter?

(4) What is the catalase activity of a liver regenerating in the body of a rat bearing a transplanted hepatic tumor?

The investigations on these questions are reported in the present paper.

## EXPERIMENTAL PROCEDURE

The rats used in this study were of the Osborne-Mendel strain and carried transplants of hepatic tumor No. 31 in the eighth, ninth, and tenth transfers (*2*). The use of this well-defined tumor material in transplants permitted an exact knowledge of the age of the transplanted tumor. The livers were removed from the animals following

decapitation of the latter and were immediately ground up with sand and extracted with distilled water as before (*1*). The diet of the animals consisted of Purina dog chow.

## Reproducibility of the Data

In the earlier report (*1*) it was stated that the reproducibility of the enzymatic determinations under comparable conditions was about 30 percent between individual animals. To a large extent this finding was attributed to the fact that the determinations were made at exactly defined concentrations of total N in the tissue extracts used. Unless this procedure is used, the variations between individual animals become extremely large. The necessity for employing some such procedure is obvious. Two samples of tissue ground and extracted separately will rarely yield exactly the same amount of extracted material. Since the enzymatic activity of the extract will be proportional to the amount of tissue extracted, it is necessary to have some knowledge of the magnitude of the latter. The most convenient standard in this connection is the amount of total N in the extract, and it has therefore been uniformly employed (*1–3*). At least two different concentrations of total N have usually been selected.

The same method of estimating the activity of catalase (*1*) was used in the present study. The activity is reported in terms of cubic centimeters of oxygen liberated per second. The volume of gas is plotted graphically against time of incubation, and the slope of the linear portion of the curve yields the reported rate. An illustration of this method is given in figure 1. The rate is obviously linear during the early stages of the reaction.

As an illustration of the reproducibility of the methods employed, the data on the livers of normal rats and of rats bearing the transplanted hepatic tumor and the Jensen sarcoma are given in table 1. The former tumor was grown in rats of the Osborne-Mendel strain, the latter tumor in Buffalo rats. The effect of the presence of the tumors on the liver catalase of the tumor-bearing animals is practically the same for both tumors.

Table 1.—*The catalase activity of the livers of rats bearing transplanted hepatic tumors 4 weeks old since the date of implantation, and of rats carrying Jensen sarcomas 3 weeks old since the date of implantation*

[Concentration of total N in each of the tissue extracts was 0.6 mg. per cubic centimeter]

| Tissue | Number of animals | Rate of oxygen evolution |
|---|---|---|
| | | *Cubic centimeters per second* |
| Livers of normal Osborne-Mendel rats. | 6 | 1.7, 1.5, 1.6, 1.8, 1.7, 1.7. |
| Livers of normal Buffalo rats. | 4 | 1.8, 1.7, 1.6, 1.5. |
| Livers of rats carrying transplanted hepatic tumor No. 31. | 6 | .12, .14, .13, .12, .11, .12. |
| Livers of rats carrying transplanted Jensen sarcoma. | 6 | .18, .17, .14, .13, .14, .15. |

In the present study several animals were employed for each determination, and in every case the reproducibility was within the range illustrated by the data in table 1. Although only one figure for each determination is given in the subsequent tables it is understood that the variation in every case is no greater than about 30 to 50 percent between the liver tissues of individual animals.

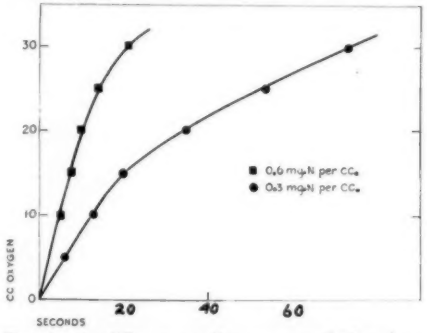


Figure 1.—The rate of oxygen evolution in a digest of liver-tissue extract and hydrogen peroxide.

### The Effect of Age of the Tumor

The data on the effect of age of the transplanted hepatic tumor on the liver catalase are given in table 2. It should be stated here that the enzymatic data reported in the earlier paper (*1*) were obtained on the tissues of animals which had borne the transplanted tumor for 28 days since the date of implantation. The time course of the tumor effect on the liver is very interesting (table 2). The effect of the tumor when the latter is 1 week old is striking, for there is a very marked increase over the normal for the liver catalase activity. The livers from eight animals at this stage were separately investigated and every one yielded the same catalase values given in table 2 within 20 percent. One week beyond this stage a marked drop below the normal level in catalase activity occurred, which continued until about the time the 4-week stage was reached, when the level, at least to 56 days, was fairly constant. The animals studied 1 week after the date of implantation all possessed definitely palpable tumors.

### The Effect of Starvation

In order to determine whether the results obtained may have been partly due to inanition, the liver catalase activity of normal and tumor-bearing rats which had been deprived of all food for 1–3 days was estimated. The starved animals received water ad libitum. The data are given in table 3.

Table 3 shows that starvation at least up to 3 days produces no appreciable effect on the liver catalase activity of the starved animals.

### The Effect of Extirpation of the Tumor Followed by Reinoculation

It is evident that the presence of the transplanted hepatic tumor in the rat results in a greatly lowered catalase activity of the liver in this animal. What will be the course of this activity if the tumor is removed, then fresh tumor material inoculated and allowed to develop, and finally this second growth of tumor removed? It might thus be possible to produce a very interesting sequence of phenomena which would throw light on certain aspects of tumor development, the possible systemic effects elicited by the presence of the tumor and by its removal, and the effect which the previous presence of a tumor may exert on the development and properties of a subsequent tumor in the same animal.

Young male rats, about 5 weeks old, of the Osborne-Mendel strain, were inoculated subcutaneously with small pieces of tissue of hepatic tumor No. 31. A number of animals bearing 4-week-old tumors were anesthetized under ether and the tumors completely removed. The fact that these tumors were encapsulated made their extirpation easy. Half of the animals so treated were killed subsequently at definite intervals, and the catalase activity of the livers was determined. The rest of the animals were reinoculated 48 hours after excision of the tumors with fresh tumor tissue, and the new tumor was allowed to grow for 4 weeks. A few of these animals

Table 2.—*The effect of the age of the transplanted hepatic tumor No. 31 on the liver catalase activity of the tumor-bearing animal*

| Days after implantation | Total N in extract | Evolution of oxygen |
|---|---|---|
| | *Milligrams per cubic centimeter* | *Cubic centimeters per second* |
| 0 | 0.6 | 1.7 |
| | .3 | .5 |
| 7 | .6 | 3.3 |
| | .3 | 1.1 |
| 14 | .6 | .7 |
| | .3 | .3 |
| 21 | .6 | .4 |
| | .3 | .2 |
| 28 | .6 | .1 |
| | .3 | .03 |
| 42 | .6 | .1 |
| | .3 | .07 |
| 56 | .6 | .1 |
| | .3 | .04 |

TABLE 3.—*The effect of starvation on rat liver catalase*

| Tissue | Age of animals or tumors [1] | Length of starvation of the animals | Total N in extract | Evolution of oxygen |
|---|---|---|---|---|
| | *Weeks* | *Hours* | *Milligrams per cubic centimeter* | *Cubic centimeters per second* |
| Normal rat livers | Animals 6 weeks old | 0 | 0.6 | 1.7 |
| | | | .3 | .5 |
| Do | do | 24 | .6 | 1.8 |
| | | | .3 | .6 |
| Do | do | 48 | .6 | 1.5 |
| | | | .3 | .5 |
| Do | do | 72 | .6 | 1.6 |
| | | | .3 | .5 |
| Livers of rats carrying transplanted hepatic tumor No. 31. | Tumors 4 weeks old; animals 9–10 weeks old. | 0 | .6 | .1 |
| | | | .3 | .03 |
| Do | do | 24 | .6 | .1 |
| | | | .3 | .02 |
| Do | do | 48 | .6 | .09 |
| | | | .3 | .03 |
| Do | do | 72 | .6 | .1 |
| | | | .3 | .04 |

[1] The age of the tumor refers to the time elapsed since implantation.

were killed in order to determine the liver catalase activity at this point. The remainder were operated upon for the removal of the tumors and were subsequently killed as described above at definite intervals, and the catalase activity of their livers was determined.

No difficulty was experienced in removing either the first or the second tumor. The animals recovered rapidly from the operation. The second inoculation of tumor into rats from which the first tumor had been removed was made when the animals were about 10 weeks old and was uniformly successful. The success of the second inoculation did not necessarily depend upon the fact that the animals had previously borne the tumor, since hepatic tumor tissue implanted into normal animals of this age will grow readily. It was observed, however, that the second tumor apparently grew somewhat more rapidly than the first and that the animals presented a somewhat emaciated appearance. The data on the livers of these animals, as well as on the tumors, are shown in table 4.

The course of the effects of the operations studied is quite clear from table 4. The livers of normal rats have a high catalase activity which is apparently independ-

TABLE 4.—*The effect of extirpation of transplanted hepatic tumor, reinoculation with fresh tumor, and extirpation of the second growth on liver catalase*

| Tissue | Age of animal or tumor [1] and time after operation | Total N in extract | Evolution of oxygen |
|---|---|---|---|
| | | *Milligrams per cubic centimeter* | *Cubic centimeters per second* |
| Normal liver | Rats 5 weeks to 1 year old | 0.6 | 1.7 |
| | | .3 | .5 |
| Livers of rats bearing transplanted hepatic tumor No. 31 | Tumors 4 weeks old | .6 | .1 |
| | | .3 | .03 |
| Livers of rats from which the transplanted tumors were removed | 24 hours after operation | .6 | 1.0 |
| | | .3 | .3 |
| | 48 hours after operation | .6 | 1.8 |
| | | .3 | .6 |
| | 72 hours after operation | .6 | 1.8 |
| | | .3 | .5 |
| | 96 hours after operation | .6 | 1.3 |
| | | .3 | .5 |
| | 168 hours after operation | .6 | 1.8 |
| | | .3 | .5 |
| Livers of rats which had been inoculated with fresh hepatic tumor tissue second time | Tumors 4 weeks old | .6 | .1 |
| | | .3 | .03 |
| Livers of rats from which the second tumor was removed | 24 hours after operation | .6 | .9 |
| | | .3 | .3 |
| | 48 hours after operation | .6 | 1.8 |
| | | .3 | .5 |
| | 96 hours after operation | .6 | 1.8 |
| | | .3 | .5 |
| First transplanted hepatic tumor | Tumors 4 weeks old | 3.0 | .01 |
| Second transplanted hepatic tumor | do | 3.0 | .01 |

[1] The age of the tumor refers to the time elapsed since implantation.

ent, within limits, of the age of the animal. The presence of a transplanted tumor in the animal causes a marked lowering in the catalase activity of the liver. Removal of the tumor results, after 24 hours, in a marked recovery of the liver catalase which is followed thereafter by the attainment of the normal level of activity of this enzyme in liver. The livers of rats bearing the second tumor have as low a catalase activity as they had when they bore the first tumor. The effect of the removal of the second tumor on the liver catalase is apparently the same as that following removal of the first tumor. The catalase activity of the first and of the second tumors is the same. It was necessary, however, to use a very much greater concentration of the tumor extract than of the liver in order to be able to determine the enzymatic activity.[1]

## Regenerating Liver in Rats Bearing the Transplanted Hepatic Tumor

We previously reported that the catalase activity of liver regenerating in normal rats was the same as that of normal liver (*1*). Will the catalase activity of regenerating liver in an animal bearing a transplanted hepatic tumor be like that of normal liver or will it be like that characteristic of nonregenerating liver in tumor-bearing animals (tables 1, 2, and 4)? The problems were investigated as before (*2*), by removing 60–70 percent of the liver of rats under ether anaesthesia. In this case, rats carrying 4-weeks-old transplanted hepatic tumor No. 31 were used. A certain proportion of the animals were killed at selected intervals after the operation, and the catalase activity of the livers was determined. Five days following removal of the lobes, the remainder of the animals were operated upon, and the tumors were removed. At selected intervals after the latter operation, the animals were decapitated, and the liver catalase activity was determined. The data are given in table 5.

The data in table 5 reveal that regenerating liver in the tumor-bearing animals has about the same order of catalase activity as that of nonregenerating liver in these animals. During the first 2 days of regeneration, the activity of the former liver is somewhat higher than that of the latter but is very much lower than that of regenerating liver or normal liver in normal animals. After the first 2 days the activity of the regenerating liver in the tumor-bearing animals is the same as that of nonregenerating liver in these animals.

TABLE 5.—*Regenerating liver in rats bearing the transplanted hepatic tumor*

| Tissue | Age[1] of rat or tumor[1] or period after removal of tumors or lobes | Total N in extract | Evolution of oxygen |
|---|---|---|---|
| | | *Milligrams per cubic centimeter* | *Cubic centimeters per second* |
| Normal rat livers | Rats 5 weeks to 1 year old. | 0.6<br>.3 | 1.7<br>.5 |
| Livers of rats carrying transplanted hepatic tumor No. 31. | Tumors 4 weeks old. | .6<br>.3 | .1<br>.03 |
| Regenerating liver from normal rats. | Rats 8 weeks old; livers regenerating 1–10 days. | .6 | 1.5–1.8;<br>.3–.5 |
| Regenerating liver from rats carrying 4-weeks-old transplanted hepatic tumor. | 24 hours after removal of lobes. | .6<br>.3 | .3<br>.1 |
| | 48 hours after removal of lobes. | .6<br>.3 | .3<br>.1 |
| | 72 hours after removal of lobes. | .6<br>.3 | .1<br>.03 |
| | 120 hours after removal of lobes. | .6<br>.3 | .1<br>.03 |
| Regenerating liver from rats carrying 4-weeks-old transplanted hepatic tumor, from which the tumors had been removed. | 1 day after removal of tumor; 6 days after removal of lobes. | .6<br>.3 | 1.0<br>.2 |
| | 2 days after removal of tumor; 7 days after removal of lobes. | .6<br>.3 | 1.6<br>.4 |
| | 3 days after removal of tumor; 8 days after removal of lobes. | .6<br>.3 | 1.8<br>.5 |

[1] The age of the tumor refers to the time elapsed since implantation.

[1] *Partial removal of the tumor results in a temporary rise in the liver catalase activity which appears to be roughly proportional to the amount of tumor removed. Only on complete excision of the tumor does the liver catalase activity return completely to the normal level.*

The removal of the tumor from rats whose livers are still regenerating causes substantially the same effect in these livers as is shown in table 4 on nonregenerating livers. The activity of the liver is almost restored after 24 hours following removal of the tumor and is wholly restored by 48 hours after the operation.

### The Thymonucleodepolymerase Activity of the Spleens of Normal and of Tumor-bearing Rats

It is of some interest to see whether the enzymatic activity of organs other than the liver are affected by the fact that the animal concerned is bearing a transplanted hepatic tumor. We mentioned briefly in an earlier report (*1*) that the catalase activity of the kidneys of rats carrying 4-week-old transplanted hepatic tumors was about one-half that of the kidneys of normal animals. It has been observed that the spleens of the tumor-bearing rats are about double the size and weight of the spleens in normal animals. This organ in either kind of rat is nearly devoid of measureable catalase activity. It has, however, marked thymonucleodepolymerase activity, and a study of this enzyme was made on the spleens from normal and from tumor-bearing rats. The method of measuring the activity of this enzyme has been described previously (*3*). The results are depicted in figure 2.

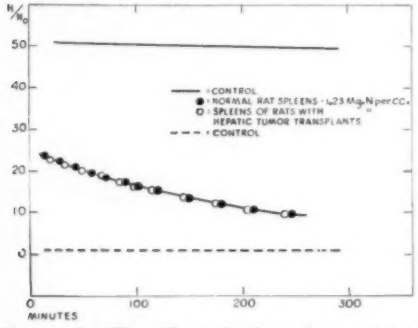


FIGURE 2.—The effect of spleen tissue extracts on thymonucleate. Ordinate, relative viscosity; abscissa, time of incubation.

It is apparent that the spleens from both sources were practically identical in thymonucleodepolymerase activity, despite the fact that these organs from the tumor-bearing animals were about twice as large and as heavy as those from normal rats.

## DISCUSSION

The greatly lowered catalase activity of the livers of rats bearing the transplanted tumors presents a phenomenon which at present is difficult to explain. That it is the presence of the tumor which causes this effect is revealed by the rapid restoration of the catalase activity within a day or two following removal of the hepatic tumor. Evidently no very deep rooted or permanent effect on the liver was produced by the presence of the tumor. The fact that the changes in the activity of the liver catalase were capable of repetition by making a second inoculation of the tumor into an animal from which the first tumor had been removed strengthens the belief that it is the presence of the tumor that produces the effect on the liver. The magnitude of this effect, as reflected by the lowering of the liver catalase activity, appears to be the same at the same stage of growth of both the first and second tumors. The apparent rate of recovery of the liver catalase following extirpation of the second tumor is the same as that following extirpation of the first tumor. Again, the catalase activity of the first and second tumors is the same. The possibility that inanition might be a contributing factor to the low liver catalase activity of the tumor-bearing animal can be ruled out (table 3). The fact that starvation from 1 to 3 days caused no appreciable effect on the liver catalase activity of either normal or tumor-bearing animals is interesting.

It is possible that the effect of the transplanted tumor on the liver catalase is elicited by a toxic substance produced in the tumor and carried by the blood to the liver. Preliminary studies on the blood catalase of tumor-bearing rats revealed no significant difference from that of normal animals (unpublished observations). However, it is likely that the toxic substance, if it exists, would be present in the blood stream at any one time in only very minute amounts, and the investigation of the blood catalase under our present conditions, therefore, would not provide a fair criterion of the presence of such a substance. In spite of the ad hoc character of the hypothesis, it is evident that some material is produced by the tumor which travels to the liver via the blood stream, and there causes by some toxic action a very marked lowering of the catalase activity. The tumor must necessarily reach a certain stage of growth in order to do this. When the tumor is removed, the catalase activity of the liver is rapidly restored to normal. If a second tumor is inoculated, the liver catalase activity again drops. When this second tumor is removed, the catalase activity is once more rapidly restored to normal. In the face of such evidence, it is difficult to avoid the idea that the tumor is directly responsible for the lowered liver catalase activity, and that its action at a distant site could only be achieved by sending material through the circulatory system to that site. The situation is somewhat analogous to postulating the presence of an enzyme whose intrinsic nature is unknown but whose presence in a tissue is assumed because of the effect it produces. Further investigations are obviously desirable and are being made at present.

It must be borne in mind, however, that certain enzyme systems in the rat liver are not affected by the presence of the transplanted hepatic tumor (*1*). The activity of amylase, xanthine dehydrogenase, and thymonucleodepolymerase is the same in the livers of tumor-bearing and normal rats. Arginase activity, however, is distinctly lowered in the livers of rats carrying the No. 31 hepatic-tumor transplant (*2*), while catalase is lowered very much more than the arginase. It would seem, granted the above toxic-substance hypothesis, that the material elaborated by the tumor will affect only certain enzyme systems, leaving others apparently untouched. It is possible, if many more enzyme systems of the liver were investigated, that other enzymes than the three above-mentioned would be found to be unaffected by the presence of the tumor transplant, while still others might be found even more drastically affected than catalase. Further studies along these lines are necessary because a wider knowledge of which enzyme systems are affected and which are unaffected would provide a clue as to the nature of the possible toxic material.[2]

The fact that the regenerating rat liver in the presence of the transplanted tumor has a catalase activity of the same order as that of nonregenerating liver in a tumor-bearing animal is not altogether surprising since the catalase activity of regenerating and normal livers in normal rats is the same (*1*). In the period of most rapid regeneration of the liver, that of the first day or two, the catalase activity of the regenerating liver in the tumor-bearing animals is not quite so low as that characteristic of nonregenerating liver and of later regenerating liver in tumor-bearing animals. It would seem as if the influence of the tumor, brought about directly by a possible blood-

[2] *We have observed that the effect of a transplanted tumor on the liver catalase is not confined to rats but occurs in several strains of mice bearing certain spontaneous and transplanted tumors. The data will be published later.*

borne toxic material, was largely, but not fully, effective on the rapidly growing liver in the early stages of regeneration. Removal of the tumor from rats whose livers were in the later regenerating period produced a restoration of the normal level of liver catalase activity similar to the results on nonregenerating liver.

The effect of age of the tumor on the liver catalase follows a striking course. The presence of a 1-week-old tumor causes the liver catalase to be almost twice as active as normal rat liver. With further growth of the tumor, there is a steady decrease in the liver catalase activity until the tumor is about 4 weeks old when the level remains nearly constant. The activation of liver catalase during the early development of the tumor is difficult to interpret at the present time, and further studies are needed to elucidate this point.

Microscopic examination of sections of liver of animals bearing the hepatic-tumor transplant No. 31 for 4 weeks after inoculation showed no deviation from the normal cell structure. This is interesting in view of the fact that the presence of the tumor at this stage caused a very marked lowering of the liver catalase activity.

## SUMMARY

The liver catalase activity of rats carrying the Jensen sarcoma at 3 weeks and of rats carrying the transplanted hepatic tumor No. 31 at 4 weeks is about one-tenth that of normal rat liver. Studies of the effect of age of the hepatic tumor revealed a marked activation of the liver catalase when the tumor was 1 week old; with increasing age of the tumor the catalase activity of the liver decreased, reaching a constant level at about 4 weeks.

Extirpation of the hepatic tumor caused a rapid restoration of the liver catalase activity which reached the normal level between 24 and 48 hours. Inoculation of a second tumor in animals from which the first tumor had been removed caused again a drop in liver catalase activity to the level produced by the presence of the first tumor at an equal stage of growth. Removal of the second tumor produced the same rate of restoration of the normal level of liver catalase activity as did removal of the first tumor. The catalase activity of the first and of the second tumor was the same.

Starvation from 1 to 3 days produced no appreciable effect on the liver catalase activity of either normal or of tumor-bearing rats.

Rat livers regenerating in animals bearing the transplanted hepatic tumor had a liver catalase activity of the same order as nonregenerating livers in animals bearing this tumor. Removal of the tumor caused a rapid restoration of the liver catalase activity to the normal level.

The effect produced by the presence of the transplanted tumor has been tentatively interpreted as being due to the elaboration by the tumor of a toxic material which travels by way of the circulatory system to the liver and there causes directly or indirectly a lowering of the catalase activity. The ramifications offered by this suggestion are discussed.

The thymonucleodepolymerase activity of the spleens of rats bearing the transplanted hepatic tumor was compared with that of the spleens of normal animals. Despite the fact that the spleens of the former are about double in size and in weight those of the latter, the activity of this enzyme in both was practically identical.

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# The Relative Enzymatic Activity of Certain Mouse Tumors and Normal Control Tissues

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## INTRODUCTION

The relative activity of a number of enzyme systems in the transplanted rat hepatic tumor No. 31 was reported previously (*1–3*). The effect of the presence of the transplanted tumor on the enzymatic activity of the livers of tumor-bearing rats was also investigated (*1, 3, 4*). It was found that the ratio of activity in the hepatic tumor to that in normal rat liver was different for each enzyme studied. Thus, the ratio for catalase was about 1 : 1,000, whereas the ratio for amylase was about 1 : 1. Of great interest was the observation that the catalase activity of the livers of rats bearing either the transplanted hepatic tumor or the Jensen sarcoma was about one-tenth that of normal rat livers.

It was considered desirable to extend enzymatic studies of this nature to a wide variety of mouse tumors for the obvious reason that any conclusions that might be drawn or hypotheses even tentatively advanced on the basis of the study of tumors in a single species of animal, would be incomplete and unwise. Earlier the relative arginase activity of a number of mouse tumors and comparable normal tissues (*1*) was reported. The present communication reports the relative activity of xanthine dehydrogenase, thymonucleodepolymerase, and catalase in a transplanted mouse lymphoma and in the spontaneous mammary tumors and transplanted hepatic tumors of two strains of mice. The normal mouse tissues for comparison are, respectively, lymph nodes and bone marrow, hyperplastic mammary tissue induced by stilbestrol, and liver. In addition the relative amylase activity of the hepatic tumors and normal mouse livers has been determined. Data have been obtained, therefore, of the enzymatic activity not only of tumors in two different species of animal, and not only of various kinds of tumors in each species, but also on several enzyme systems in each tumor.

Finally, some preliminary data are reported on the catalase activity of the livers of mice carrying certain tumors. Since this particular study forms part of an extended investigation which will be reported in detail in a subsequent paper on the possible systemic effects of spontaneous, induced, and transplanted mouse tumors, only a brief mention is made of some of the results obtained thus far.

## EXPERIMENTAL PROCEDURE

The various tissues studied were extracted and the enzymatic activity of the extracts determined, exactly as described earlier for the rat tissues (*3*). In every case the tissue used was removed from the animal immediately after death. The tissue was ground to a paste, taken up in three times its weight of distilled water, and the mixture allowed to stand at 8° C. for 18 hours. The extract was then centrifuged and the total N determined in an aliquot of the supernatant. For each enzy-

matic determination, the supernatant was diluted with distilled water to a definite N concentration. The reasons for employing tissue extracts on this concentration basis were extensively discussed in a previous report (*4*). It need only be added that when this procedure is used, the differences in enzymatic activity of the same tissue in different mice are rarely greater than 50 percent and are frequently less. The variability in mice is somewhat greater than in rats, for in tissues of the latter animals we found variations in enzymatic activity of individual rats to be rarely greater than 30 percent. In any event, the tissues from several animals were used in the present study for each series of enzymatic determinations.

The procedures for the enzyme studies were described in detail in earlier reports (*2, 3, 4*) and were followed exactly in the present studies.

### Thymonucleodepolymerase

The activity of thymonucleodepolymerase was studied in the transplanted hepatic ( I × dilute brown and C3H) tumors and in the transplanted lymphoma No. 72942 (strain A).[1] Normal mouse tissues used for comparison were, respectively, liver, and lymph nodes and bone marrow. The enzymatic technique has been described (*2*). The hepatic tumor was in the $F_1$ generation of the hybrid mice.

The results on the mouse hepatic tissues are given in figure 1.

There is apparently a marked difference between the activity of the hepatic tumors in the two strains of mice. The depolymerase activity of the C3H mouse tumor is much greater than that of normal liver, whereas the activity of the I × dilute brown mouse tumor is somewhat weaker. We have found no difference in the depolymerase activity of the normal livers of these two strains. The difference in activity, however, between the transplanted hepatic tumors in the two strains is very great.

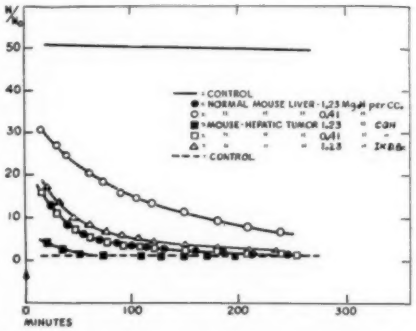


FIGURE 1.—Thymonucleodepolymerase activity of mouse hepatic tissues. Ordinate, relative viscosity; abscissa, time of incubation.

In figure 2 are given the results on the mouse lymphatic tissues.

The most striking observation in the case of the lymphatic tissues is the extremely high depolymerase activity of normal mouse lymph nodes. The latter tissue possesses by far the greatest activity of any of the mouse or rat tissues studied

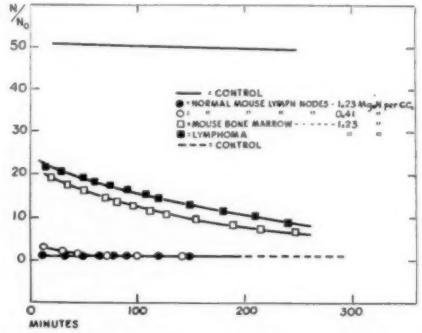


FIGURE 2.—Thymonucleodepolymease activity of mouse lymphatic tissues. Ordinate, relative viscosity; abscissa, time of incubation.

[1] *A brief description of the hepatic tumors was given earlier* (1). *The hepatic tumors in C3H mice were kindly furnished by Dr. J. W. Thompson.*

to date. The lymphoma is very much weaker in activity than the lymph nodes and is slightly weaker than normal bone marrow.

### Amylase

The amylase activity of normal mouse liver and of the transplanted hepatic tumors from C3H and from I × dilute brown mice was determined as before (*3*). The data are collected in table 1.

The data in table 1 reveal that the amylase activity of the two transplanted hepatic tumors is quite different. The activity of the tumor in C3H mice is greater than that of either normal mouse liver or of the tumor in I × dilute brown mice. The amylase activity of the latter two tissues appears to be about the same. The amylase activity of the normal livers of both strains is also the same.

### Xanthine Dehydrogenase

The activity of xanthine dehydrogenase was determined as heretofore (*3*) on the hepatic, mammary, and lymphatic mouse tissues described above. The data are given in table 2.

Table 1.—*Amylase determination* [1]

[Incubation temperature 39° C.]

| Tissue | Concentration of extract | Time of incubation | 0.02 N thiosulfate |
|---|---|---|---|
| | *Milligrams N per cubic centimeter* | *Hours* | *Cubic centimeters* |
| Transplanted hepatic tumor in C3H mice | 2.6 | 1 | 3.1 |
| | | 2 | 4.0 |
| | | 4 | 4.6 |
| | 1.3 | 1 | 2.4 |
| | | 2 | 3.0 |
| | | 4 | 3.9 |
| Transplanted hepatic tumor in I × dilute brown mice | 2.6 | 1 | 1.7 |
| | | 2 | 2.8 |
| | | 4 | 4.0 |
| | 1.3 | 1 | 1.2 |
| | | 2 | 2.1 |
| | | 4 | 3.1 |
| Normal mouse livers | 2.6 | 1 | 1.6 |
| | | 2 | 2.9 |
| | | 4 | 4.1 |
| | 1.3 | 1 | 1.3 |
| | | 2 | 2.1 |
| | | 4 | 3.2 |

[1] Data in the last column represent the difference in titer of the mixture with and without added glycogen minus the value due to the original reducing groups of the glycogen.

Table 2.—*Xanthine dehydrogenase activity at 25° C.*

[Test: 1 cc. tissue extract+1 cc. M/5 phosphate buffer of pH 6.9+1 cc. xanthine solution (0.0016 M)+1 cc. methylene blue (0.00124 M). Control: Same as test except 1 cc. water substituted for the substrate. In every case more than 5 hours required for decolorization.]

| Tissue | Total N in extract | Time of decolorization |
|---|---|---|
| | *Milligrams per cubic centimeter* | *Minutes* |
| Transplanted hepatic tumor in C3H mice | 2.6 | 38 |
| | 1.3 | 60 |
| Transplanted hepatic tumor in I × dilute brown mice | 2.6 | 10 |
| | 1.3 | 25 |
| Normal mouse liver | 2.6 | 10 |
| | 1.3 | 25 |
| Spontaneous mammary tumors in C3H mice | 2.6 | 30 |
| | 1.3 | 75 |
| Hyperplastic mouse mammary tissue induced by stilbestrol | 2.6 | 45 |
| | 1.3 | 113 |
| Transplanted lymphoma No. 72942 in A mice | 2.6 | 25 |
| | 1.3 | 48 |
| Normal mouse lymph nodes | 2.6 | 240 |
| | 1.3 | >300 |
| Normal mouse bone marrow | 2.6 | 45 |
| | 1.3 | 100 |

Table 2 reveals the considerable difference in the enzymatic activity of the transplanted hepatic tumors in the two strains of mice. The xanthine dehydrogenase activity of the tumor in the I × dilute brown strain is identical with that of normal mouse liver. On the other hand, the activity of the tumor in C3H mice is much less than that of either of the former two tissues.

The xanthine dehydrogenase activity of the spontaneous mammary tumor is slightly greater than that of hyperplastic mammary tissue. The activity of the transplanted lymphoma is very much greater than that of normal lymph nodes, and about twice as great as that of normal mouse bone marrow.

### Catalase

The activity of catalase in hepatic, mammary, and lymphatic tissues of the mouse was determined as described earlier (*3*, *4*). The data are collected in table 3.

Table 3 shows the considerable difference in enzymic activity between the transplanted hepatic tumors in the two strains of mice. The catalase activity of the tumor

TABLE 3.—*Catalase activity of mouse tumors and normal control tissues*

[Temperature 27° C. 1.0 cc. of 29 percent hydrogen peroxide added to mixture of 1.0 cc. extract plus 5 cc. of M/5 phosphate buffer at pH 6.9.]

| Tissue | Concentration of extract | Rate of oxygen evolution |
|---|---|---|
| | *Milligrams N per cubic centimeter* | *Cubic centimeters per second* |
| Transplanted hepatic tumors in C3H mice | 3.0 | 0.25 |
| | 1.5 | .10 |
| Transplanted hepatic tumors in I × dilute brown mice | 3.0 | 3.3 |
| | 1.5 | 1.9 |
| | .6 | .4 |
| | .3 | .2 |
| Normal livers in C3H mice | .6 | 2.2 |
| | .3 | .7 |
| Normal livers in I × dilute brown mice | .6 | 2.4 |
| | .3 | .8 |
| Spontaneous mammary tumors in C3H mice | 3.0 | .09 |
| | 1.5 | .04 |
| Spontaneous mammary tumors in A mice | 3.0 | .09 |
| | 1.5 | .05 |
| Hyperplastic mouse mammary tissue induced by stilbestrol | 3.0 | .09 |
| | 1.5 | .04 |
| Transplanted lymphoma No. 72942 in A mice | 3.0 | .04 |
| | 1.5 | .02 |
| Normal mouse lymph nodes | 3.0 | .09 |
| | 1.5 | .05 |
| Normal mouse bone marrow | 3.0 | .06 |

in C3H mice is much less than that in I × dilute brown mice. Both tumors are in turn much less active than normal mouse liver.

In contrast with that of the hepatic tissues, the catalase activity of the spontaneous mammary tumors in two different strains of mice, as well as that of the hyperplastic mammary tissue, is practically the same for all three tissues.[2] The activity of the transplanted lymphoma is distinctly less than that of either normal lymph nodes or bone marrow. In general, it appears that the catalase activity of the mammary and the lymphatic tissues is much lower than that of hepatic tissues.

## PRELIMINARY STUDIES OF THE CATALASE ACTIVITY OF THE LIVERS OF MICE CARRYING SPONTANEOUS AND TRANSPLANTED TUMORS

We have observed that the liver catalase activity of mice bearing the following tumors is considerably lower than that of normal mouse liver: transplanted hepatic tumors in I × dilute brown mice, spontaneous mammary tumors in both C3H and A strains, lymphoma No. 72942 in A mice, hemangiomas in C mice, and sarcomas S37 and CR180 in dilute brown mice. In the case of the spontaneous mammary tumors, no change from the normal in the liver catalase activity was noted until the tumors were about 5 to 6 weeks beyond the point when they were first palpable. The lowering of the liver catalase activity was most marked in animals carrying the S37 tumor. Tumors which apparently did not affect in any way the normal level of liver catalase in the tumor-bearing animals included the transplanted hepatic tumor in C3H mice, the transplanted intestinal tumor in A mice, and the transplanted melanoma in dilute brown mice. The normal liver catalase activity of each of the above-mentioned strains was determined and found to be practically the same for all. Complete details of these and of investigations at present being conducted along this line will be presented in subsequent papers.

[2] *The strain A spontaneous mammary tumor was kindly furnished by Dr. Heston.*

## DISCUSSION

The advisability of studying several enzyme systems in each type of tumor studied is clearly revealed by the data obtained in this and earlier investigations. Where one enzyme will be more active in the tumor than in the normal control tissue, another may be either equally or less active. The direction in activity which a normal tissue takes when it becomes malignant cannot be defined in terms of any single enzyme system. As stated earlier (*3*), it seems quite likely that the enzyme systems of a particular normal tissue are affected in varying degrees by the alteration of the tissue into a tumor. Thus it has been shown in the comparison of normal rat livers and rat hepatic tumors that whereas the catalase activity of the

tumor is about a thousand times less than that of normal liver, the amylase activity of the two tissues is the same (*3*). Similar results have been obtained in the study of the mouse tumors reported in this paper and have extended the field beyond that developed by the earlier studies.

### Transplanted Hepatic Tumors

With one exception, the activity of the enzymes in the tumors in the C3H and the I × dilute brown mice appear to be different. The exception is arginase, which has been reported to be equally active in transplanted hepatic tumors of C3H and of I mice (*1*). Lest some doubt arise as to the advisability of considering the arginase activity of tumors in I mice in connection with the series of studies on tumors in the I × dilute brown hybrids, we determined the arginase activity of the transplanted hepatic tumor in the latter animals and found it to be identical with that of the former (unpublished observations). It should be mentioned that the arginase activity of the transplanted tumors is extremely low as compared with that of normal livers (*1*).

The thymonucleodepolymerase activity of the transplanted hepatic tumor in rats is slightly lower than that of normal rat liver (*2*). In tumors of C3H mice, this enzyme is much more active than in normal mouse liver; in tumors of I × dilute brown mice, the activity is slightly less than that of normal liver. In rats, the tumor amylase activity is equal to that in normal liver. In C3H mice the tumor amylase activity is much greater than in normal liver whereas in I × dilute brown mice the activity of the tumor and of normal liver is the same. In rats and in C3H mice the tumor xanthine dehydrogenase is lower than that in the corresponding normal livers; in I × dilute brown mice the activity is the same as that of normal mouse liver. Finally, in the transplanted hepatic tumor in both rats and the two strains of mice, the catalase activity, like the arginase activity, is very much lower than that of the respective normal livers of each species. In connection with the comparison of the catalase activity of the hepatic tissues in both species of animals, it is interesting to note that the differences in activity between liver and tumor is very much greater in the rat than in the mouse. The activity, however, of each enzyme is greater in mouse than in comparable rat tissues.

The differences in enzymic activity of the hepatic tumors in the two strains of mice is difficult to interpret at the present time. A further difference between the two tumors is revealed by the activity of the liver catalase of animals of each strain bearing the respective tumors, for while the activity of the C3H mouse livers remains at the normal level, the activity of the I × dilute brown mouse livers is much lower than that of the livers of normal mice. The tumor in the C3H mice is very slow growing. It was studied after it had grown about 9 months since the date of implantation. On the other hand, the tumor in the I × dilute brown mice grows very quickly, and it was investigated about 2 weeks since the date of implantation. Further studies are obviously needed to clarify the observations on tumors with such different rates of growth, and investigations along this line are proceeding at the present time.

### Spontaneous Mammary Tumors

It was reported that both the arginase activity (*1*) and the depolymerase activity (*2*) of the spontaneous mammary tumors in mice were greater than that of hyperplastic mammary tissue induced by subcutaneous stilbestrol administration. The

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results described in the present paper indicate that while the same conclusion may be drawn in the case of xanthine dehydrogenase, the catalase activity of the mammary tumor and of the hyperplastic breast is practically the same. The catalase activity of the spontaneous tumors in both the C3H and the A strains was the same; the arginase activity of the spontaneous mammary tumors in the C3H strain and in the C57 × A hybrids was also the same (*1*).

### Transplanted Lymphoma No. 72942

In the comparison of the enzymic activity of this tumor with that of normal lymph nodes there is again encountered, as in the case of the hepatic and the mammary tumors, relatively small differences between certain enzyme systems and enormous differences between others. The arginase activity of this tumor is nearly the same as that of normal lymph nodes (*1*). The xanthine dehydrogenase activity in the tumor is considerably greater than in the nodes or in normal bone marrow. On the other hand, the depolymerase activity and the catalase activity in the tumor are both considerably weaker than in the normal nodes or in normal bone marrow.

## SUMMARY

The activity of thymonucleodepolymerase, amylase, xanthine dehydrogenase, and catalase were determined in transplanted hepatic and lymphatic tumors and in spontaneous mammary tumors in mice. The activity of these enzymes were compared with that derived from normal control tissues, respectively, liver, lymph nodes and bone marrow, and hyperplastic breast. Previously reported data on the activity of these enzymes in normal and tumor tissues of the rat, as well as earlier data on the arginase activity of both rat and mouse tissues, were used for comparison.

The following enzymes had very nearly the same activity in the tumor and in the comparable normal tissue: arginase in mouse lymphatic tissue, amylase in rat hepatic and in I × dilute brown mouse hepatic tissues, xanthine dehydrogenase in I × dilute brown mouse hepatic tissues, and catalase in mouse mammary tissues. The catalase and the arginase activity of the hepatic tumors in rats and in mice was invariably lower than in the normal livers of each species. The depolymerase activity of the rat and I × dilute brown mouse hepatic tumors and of the mouse lymphoma was lower and that of the C3H mouse hepatic tumor and of the spontaneous mammary tumor was higher than that determined in the comparable normal control tissues. The xanthine dehydrogenase activity of the rat and the C3H mouse hepatic tumors and of the spontaneous mammary tumor was lower and that of the mouse lymphoma was higher than in the comparable normal control tissues. The catalase activity of the lymphoma, the arginase activity of the spontaneous mammary tumors, and the amylase activity of the C3H mouse hepatic tumor was greater in each case than the activity of the corresponding normal control tissue.

Although the transplanted hepatic tumors in the C3H and in the I × dilute brown mice have the same arginase activity, they differ considerably in the activity of all the other enzymes studied. The two tumors have markedly different rates of growth.

A comparison of the activity of each enzyme in comparable rat and mouse tissues revealed the relatively greater activity in the mouse tissues.

A preliminary report is made of the liver catalase activity of mice bearing transplanted and spontaneous tumors.

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# Ribonuclease and Thymonucleodepolymerase

By JESSE P. GREENSTEIN, *research fellow*, and WENDELL V. JENRETTE, *junior physiologist*, *National Cancer Institute, National Institute of Health, United States Public Health Service*

Until comparatively recently it was believed that ribonucleic or yeast nucleic acid was found only in plants, whereas desoxyribosenucleic acid or thymonucleic acid was found only in animal tissues. This viewpoint has changed because of the following: (1) The isolation of ribosenucleic acid from the pancreas (*1*); (2) the isolation in crystalline form of ribonuclease from the pancreas (*2*); and (3) the detection of desoxyribosenucleic acid in plants (*3–5*). The problem of the distribution of the two types of nucleic acid has been further defined by assigning desoxyribosenucleic acid to the nuclei of both plant and animal tissue and ribosenucleic acid to the cytoplasm (*3–5*).

We reported earlier the presence of an enzyme in animal tissues which depolymerizes desoxyribosenucleic acid (*6*). To this enzyme was given the name thymonucleodepolymerase. It occurred to us that if plant tissues contain desoxyribosenucleic acid, it is probable that they also contain the appropriate depolymerase. In order to test this possibility, thymonucleic acid was incubated with extracts of the germ of several varieties of plant seeds. The results of this study are reported herein.

Of more fundamental interest, however, is the question of whether the enzyme which depolymerizes desoxyribosenucleic acid is identical with the enzyme which depolymerizes ribosenucleic acid. It is possible to suggest a satisfactory answer to the problem by the use of crystalline ribonuclease, an enzyme isolated from beef pancreas by Kunitz (*2*). This enzyme rapidly depolymerizes ribosenucleic acid. Through the kindness of Dr. Kunitz we obtained an amount of the crystalline enzyme and studied its effect on desoxyribosenucleic acid. For supplemental information we investigated the thymonucleodepolymerase activity of extracts of beef pancreas and of calf thymus.

The extracts of the seed germs and of the animal tissues were prepared in the same fashion as that described earlier (*6*). In every case, the extract after careful centrifugation was made up to a definite concentration, described in milligrams of total N per cubic centimeter. The crystalline ribonuclease dissolved readily in water and was made up in several concentrations. These solutions served as the enzyme sources. The substrate as before was sodium thymonucleate (preparation No. 2 (*7*)), made up in 1-percent aqueous solution. After equal volumes of enzyme and substrate were mixed, an aliquot of the mixture was transferred to a viscometer and the rate of viscosity was measured. The external pressure was held constant at 16 cm. water, the incubation temperature was 30° C., and the pH was 6.9 to 7.0. A complete description of the method used for this kind of study, together with an explanation of the graphical representation of the data, is given in the previous paper (*6*).

The data for the effect of the plant-germ extracts on thymonucleate are given in figure 1. The germs of the seeds of the lima bean, sunflower, wheat, corn, and pumpkin were used. It is clear that all of the extracts studied contain a factor which

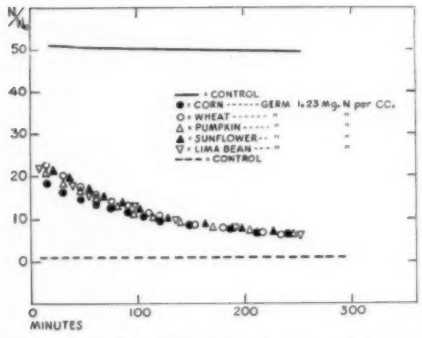


FIGURE 1.—The effect of plant-germ extracts on thymonucleate. Ordinate, relative viscosity; abscissa, time of incubation.

depolymerized thymonucleate. All the extracts appear to have nearly equal enzymic activity.

The data for the effect of crystalline ribonuclease, as well as those of the animal-tissue extracts, are given in figure 2. The ribonuclease up to a concentration as high as 8 mg. of protein per cubic centimeter had no effect upon thymonucleic acid other than that shown by any inert protein (*6*). The effect of such proteins as crystalline egg and horse-serum albumin is to decrease the viscosity and streaming birefringence of thymonucleate to a value in proportion to the concentration of protein used. After this initial drop, however, there is no further lowering of these properties with time, such as occurs in tissues which contain a depolymerizing factor. As before (*6*), a careful distinction should be made between enzymatic depolymerization, which is a dynamic process leading ultimately to a complete breakdown of thymonucleic acid to its constituent nucleotides, and inert protein or salt depolymerization, which after an initial practically instantaneous drop in the degree of thymonucleate polymerization remains at a constant level indefinitely. The rate at which the relative viscosity of the digestion mixtures in the former process approaches the value of water is a measure of the enzymatic activity of the tissue. The stationary degree of fall in viscosity in the latter process is a function only of the concentration of inert agent and the nature of the chemical groupings of the latter.

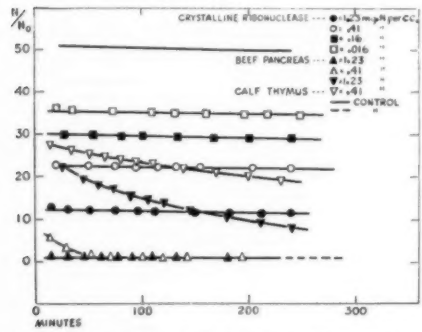


FIGURE 2.—The effect of ribonuclease and of tissue extracts on thymonucleate. Ordinate, relative viscosity; abscissa, time of incubation.

Toward thymonucleic acid, therefore, ribonuclease behaves as an inert protein.[1] On the other hand, it is evident that the extract of beef pancreas, a tissue in which the crystalline ribonuclease is found in relatively high amounts, shows very great thymonucleodepolymerase activity. The latter is illustrated by the fact that within 15 minutes the relative viscosity of the mixture dropped from about 50 to little more than unity (fig. 2). The activity of extracts of calf thymus has been included because this tissue was the source of the substrate studied. The depolymerase activity of the thymus is considerably less than that of the pancreas.

The present data reveal the presence of a depolymerizing factor in plant tissues for thymonucleic acid. The possibility of the occurrence of the latter in plant tissues is

[1] *This preparation of ribonuclease was highly active in depolymerizing yeast nucleic acid, as revealed at very low concentrations of the enzyme.*

thereby strengthened and confirms the contemporary view that no distinction should be made between plant and animal nucleic acid. If desoxyribosenucleic acid is confined to the nuclei of plant and animal cells (*3–6*), it seems highly probable that the enzyme which affects its state of aggregation must also exist exclusively in the nuclei.

The lack of any enzymatic effect of crystalline ribonuclease on thymonucleic acid indicates that each type of nucleic acid requires its own specific enzyme for depolymerization and, as a corollary, that the molecular structure of each nucleic acid is fundamentally different. That this difference in enzyme effect is not necessarily due to the differences in degree of polymerization of the two nucleic acids is suggested by the fact that ribonuclease in a concentration sufficiently high to reduce the viscosity of a thymonucleate mixture to one-fifth of the original value and hence the degree of polymerization by roughly a comparable amount, still does not exert any enzymatic activity. The highly active thymonucleodepolymerase effect of the pancreas, from which ribonuclease is isolable, also indicates that a factor differing from the ribonuclease is present. Further study, however, is desirable, particularly with a view toward isolating the thymonucleodepolymerase in purified form. Efforts in this direction are being made at present.

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# Note on the Composition of the Nucleoprotein Fraction of Normal Liver and of the Transplanted Hepatic Tumor in the Rat

By JESSE P. GREENSTEIN, *research fellow*, WENDELL V. JENRETTE, *junior physiologist*, and JULIUS WHITE, *research fellow, National Cancer Institute, National Institute of Health, United States Public Health Service*

When a normal tissue becomes malignant, it is of considerable interest in the chemical characterization of such a process to know whether changes in the general composition of the former tissue occur. As an example of this type of study we chose to investigate the composition of the nucleoprotein fraction of the following pair of comparable tissues: normal rat liver and transplanted hepatic tumor No. 31 in rats of the Osborne-Mendel strain. In an earlier report, the preparation and composition of this protein fraction from normal liver, as well as the reasons for its study, were described (*1*). Since then a considerable quantity of the tumor transplants has been made available, and in the present communication a brief report of the composition of the comparable fraction from this tissue is given.[1]

The preparation of the nucleoprotein fraction from the hepatic tumor followed exactly the method described earlier for the normal liver tissues (*1*). The physical properties of this fraction appeared to be identical with those from the normal tissue. The yield of dried protein was about 0.6 percent of the wet weight of the tissue and thus somewhat lower than the yield from normal liver tissue. Like the protein from the latter source, the tumor nucleoprotein was a light tan, nonhygroscopic powder. The analyses of this material were carried out as earlier described, and for comparison the values for the protein from normal rat liver are included in table 1 (*1*).

The tumor nucleoprotein preparation contained fat, which was removed and analyzed as before (*1*). The comparable data are given in table 2.

It is apparent from tables 1 and 2 that the corresponding nucleoprotein fractions from normal rat liver and from the transplanted hepatic tumors are nearly identical in the proportion of certain amino acids. This finding must be contrasted with the

[1] *We are indebted to Dr. J. W. Thompson for much of this material.*

TABLE 1.—*Analytical values for the nucleoprotein preparations* [1]

| Source | N | Amide N | P | Total S | Free —SH in native protein as cysteine | Free —SH in denatured protein as cysteine | Cystine-cysteine | Methionine | Tyrosine | Tryptophane |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Percent* | *Percent* | *Percent* | *Percent* | *Percent* | *Percent* | *Percent* | *Percent* | *Percent* | *Percent* |
| Normal rat liver | 15.6 | 0.9 | 0.82 | 1.19 | 0.2 | 0.2 | 1.4 | 3.0 | 3.8 | 1.5 |
| Rat hepatic tumor No. 31 transplants | 15.5 | .9 | .70 | 1.05 | .2 | .2 | 1.4 | 2.9 | 3.6 | 1.5 |

[1] Preparations previously treated with hot alcohol-acetone-ether and dried subsequently at room temperature *in racuo*.

TABLE 2.—*The proportion of fat in the nucleoprotein preparations*

| Source | Total fat | Nitrogen in fat | Phosphorus in fat | P/N ratio [1] |
|---|---|---|---|---|
| | *Percent* | *Percent* | *Percent* | |
| Normal rat liver | 14.8 | 0.8 | 2.1 | 2.6 |
| Rat hepatic tumor No. 31 transplants | 15.0 | .8 | 2.0 | 2.5 |

[1] The P/N ratio for phospholipids is 2.8.

data derived from studies of the relative enzymatic activity of these two tissues whereby striking differences in certain enzyme systems were revealed (*2*, *3*, *4*).

It would be wiser, however, not to carry this finding of the identity of composition of the two nucleoprotein fractions very far in the realm of speculation. First of all, this fraction, while obviously of importance, constitutes only about 5 percent of the total proteins in each type of tissue. Secondly, while the partial composition of these nucleoproteins may be similar, it is possible that other properties such as the relative state of aggregation or the steric configuration may be different. Thirdly, it may be pointed out that the composition of the nucleoprotein fraction of the Jensen rat sarcoma (*5*) is very similar to that of the two comparable protein fractions under consideration, as well as to the same fractions from the livers of such widely different species as the cow and the rabbit (*1*). It is possible, therefore, that the same nucleoprotein fractions obtained by the present method from various animal tissues may have nearly similar compositions. This possibility is supported by the work of Bailey (*6*) who showed that the myosins of a number of unrelated species had substantially the same composition. Much more work is needed to settle this point. While the matter belongs obviously to the field of comparative biochemistry, it nevertheless illustrates how dependent interpretation in cancer research may be on the development of collateral fields.[2]

[2] *As a result of the work to date on the comparative chemistry of the proteins Lugg has stated* (7), "*it would seem in general that the proteins of animal tissues having similar functions will have similar physical properties and will be similar in composition.*"

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# Note on the Transfer of the Strain C3H Milk Influence Through Successive Generations of Strain C Mice

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It was shown (*1*) that the foster nursing of low-tumor-strain mice by high-tumor-strain females produced a definite increase in the incidence of mammary tumors in the fostered mice. The most striking results were obtained with breeding females of strain C which have an incidence of less than 2 percent of mammary tumors but which, when fostered by high-tumor-strain C3H females, exhibit a mammary-tumor incidence of 64 percent at an average age of 8.1 months. This finding showed that the mammary glands of strain C mice are highly susceptible to the cancer-inciting influence in the milk of strain C3H females. The question arose as to whether strain C mice which are fostered by strain C3H females can transfer the milk influence to their young.

An investigation was begun during January 1940 by transferring strain C mice, within 17 hours after birth, to strain C3H foster mothers. When the young were weaned, they were designated as the F generation; and they represent the only strain C mice in this study which obtained any milk from or had any contact with strain C3H mice.

Mice of the F generation were mated brother to sister to procure the $F_1$ generation, and all subsequent generations were obtained by brother-to-sister breeding only.

The results up to October 1, 1941, are presented in table 1. Each female of the fostered F generation bore 3 litters; 1 litter was raised, and 2 litters were killed within 24 hours after birth. Of 20 mice in this generation, 14, or 70 percent, developed mammary tumors, and 4 were living and without tumors on that date.

The $F_1$ generation females bore 1, 2, or 3 litters. Of a total of 61 mice in this generation, 50, or 80 percent, developed mammary tumors, and 9 were living and free from tumors.

The $F_2$ generation females were divided into 3 groups; 1 was kept as virgins, 1 group bore 1 litter, and 1 bore 2 litters. Of 26 virgins, none has developed a tumor

Table 1.—*The occurrence of mammary-gland tumors in fostered strain C female mice and in two generations of their descendants*

| Generation | Number of litters born | Number of mice | Number developing mammary tumor | Tumor incidence | Average tumor age | Number dying without mammary tumor | Average age at death | Number living without mammary tumor, Oct. 1, 1941 | Average age of living mice |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *Percent* | *Months* | | *Months* | | *Months* |
| F | 3 | 20 | 14 | 70 | 9.3 | 2 | 16.0 | 4 | 19.7 |
| $F_1$ | 1 | 4 | 3 | 75 | 10.0 | 0 | ..... | 1 | 14.5 |
| $F_1$ | 2 | 9 | 9 | 100 | 8.1 | 0 | ..... | 0 | ..... |
| $F_1$ | 3 | 48 | 38 | 79.1 | 8.5 | 2 | 9.0 | 8 | 14.6 |
| $F_2$ | 0 | 26 | 0 | 0 | ..... | 1 | 4.0 | 25 | 9.5 |
| $F_2$ | 1 | 93 | 20 | 21.5 | 7.9 | 3 | 6.3 | 70 | 9.2 |
| $F_2$ | 2 | 40 | 26 | 65 | 7.6 | 2 | 8.5 | 12 | 9.9 |

at an average age of 9.5 months. Of 133 breeding mice, 46, or 34.6 percent, developed mammary carcinomas; and 82, or 61.6 percent, were living and free from tumor on October 1, 1941.

The primary purpose in reporting the findings up to the present time is to record the transfer of strain C3H milk influence through two generations of strain C mice. Bittner (*2*) published data indicating that if a small amount of milk is ingested by new-born mice of strain A it may produce tumors in them and in their descendants but that "the tumor incidence for the descendants of these cancerous fostered females decreased with each generation." His findings indicate that the milk influence decreases in activity in subsequent generations of susceptible mice and suggests that it is not capable of self-reproduction. Bittner's results were obtained with high-mammary-tumor strain A mice while the findings reported here were obtained with low-mammary-tumor strain C mice. Future generations of strain C animals should reveal whether the extrachromosomal influence also decreases in activity in a low-tumor strain.

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